Universidade de Aveiro
2013

Secção Autónoma de Ciências da Saúde

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Preparation and Handling of Investigational Medicinal Products

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Projeto apresentado à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biomedicina Farmacêutica, realizado sob a orientação científica do Dr. Ricardo Lima, Chefe do Laboratório de Desenvolvimento Farmacêutico de BIAL e co-orientação do Doutor Bruno Gago, Professor Assistente Convidado e Vice Diretor do Mestrado em Biomedicina Farmacêutica da Secção Autónoma de Ciências da Saúde da Universidade de Aveiro

À memória de António da Silva Sequeira

o júri

presidente

Professor Doutor Nelson Fernando Pacheco da Rocha
Professor Catedrático da Universidade de Aveiro

Professor Doutor Samuel Martins Silvestre
Professor Auxiliar da Faculdade de Ciências da Saúde da Universidade da Beira Interior

Professor Doutor Bruno Miguel Alves Fernandes do Gago
Professor Auxiliar Convidado da Universidade de Aveiro

agradecimentos

A todos que contribuíram para que este momento se tornasse realidade e que estiveram ao meu lado durante a realização deste projeto, deixo o meu profundo agradecimento.

Ao Dr. Ricardo Lima, orientador deste trabalho, por quem nutro um grande respeito e admiração, pelos seus ensinamentos que são para mim uma fonte de inspiração.

Ao Professor Doutor Bruno Gago, co-orientador deste trabalho, pela forma atenta com que acompanhou o desenrolar do mesmo, pelas palavras de incentivo, pela paciência e apoio demonstrados ao longo deste percurso.

Aos meus colegas de mestrado, sobretudo à Ana Teresa que sempre me apoiou e que foi testemunha desta fase. Aos momentos de partilha, ao carinho e aos conhecimentos que me transmitiu.

Aos meus colegas de trabalho, sobretudo à Rita que contribuiu com um grande incentivo para que eu concretizasse este trabalho e para seguir em frente com mais projetos.

A todos os meus amigos por me suportarem até nos dias mais cinzentos, principalmente à Lena e à Fabiana. Obrigada por continuarem sempre aí.

Ao Victor, pelo companheirismo e pelo apoio incondicional. Pelas palavras de conforto e incentivo permanente. Por me ensinares que afinal os pequenos nadas da vida são aqueles que valem. Acima de tudo, pela amizade sincera que possuímos.

Aos meus pais, os meus grandes pilares permanentes, por terem sempre acreditado que eu seria capaz. Por não me deixarem desanimar ao longo deste período. Obrigada por estarem sempre presentes na minha vida e por e terem ajudado a ultrapassar os maus momentos. Vocês são os meus ídolos!

palavras-chave

Medicamento experimental, ensaio clínico, pré-formulação, formulação, bioequivalência, formas farmacêuticas, boas práticas de fabrico, boas práticas clínicas, logística, dispensa, sistemas interactivo de resposta de voz, sistema interativo de resposta web.

resumo

Face a todos os desafios relacionados com a produção e logística do medicamento experimental, este projeto tem como objetivo fornecer uma visão geral sobre o desenvolvimento de um medicamento experimental, assim como os principais detalhes que devem ser considerados na preparação, embalagem, rotulagem e distribuição de medicamentos experimentais, de forma a fornecer uma ferramenta de referência rápida em contexto profissional e académico.

Foi realizada uma revisão na literatura internacional para identificar estudos com foco no desenvolvimento e distribuição de medicamentos experimentais, principalmente através de bases de dados como o PubMed e Medline, embora também se tenha recorrido a *guidelines,* e alguns livros e teses de mestrado e doutoramento sobre o objeto de estudo. Foram selecionados apenas artigos na língua inglesa. Sempre que possível, foram selecionados os artigos mais recentes.

Durante o processo de desenvolvimento de um medicamento experimental, os estudos de solubilidade, assim como os de biodisponibilidade e bioequivalência, são os maiores desafios nas fases iniciais de desenvolvimento. Atendendo à produção do medicamento experimental, aspectos críticos, como os comparadores, os processos de *blinding* e embalamento, são determinantes para o sucesso de todo o ensaio clínico. Após a completa preparação do medicamento experimental, este é introduzido nas diferentes fases de ensaio clínico, com o objetivo de fornecer uma ampla informação, como a eficácia e a segurança. Todo este processo deve englobar uma série de requisitos previamente estabelecidos, assim como uma equipa devidamente treinada, de forma a minimizar os custos associados ao desenvolvimento do medicamento experimental, acelerando a sua entrada no mercado.

É importante ressalvar que embora exista uma ampla gama de informação sobre a preparação de medicamentos experimentais, foi encontrada pouca informação sobre a logística, sendo fundamental, no futuro, explorar mais a temática da distribuição de medicamentos experimentais, abordando os principais resultados não satisfatórios das auditorias, no âmbito dos ensaios clínicos, e encontrar ferramentas e procedimentos para evitar as não-conformidades.

keywords

Investigational medicinal product, clinical trial, pre-formulation, formulation, bioequivalence, drug delivery systems, good manufacturing practice, good clinical practice, logistics, dispensing, interactive voice response systms, interactive web response system.

abstract

Duo to all of the challenges related with the production and logistics of the investigational medicinal products, this project aims to make an overview about the development of an investigational medicinal product, and the main details that must be considered in the preparation, packaging, labelling and distribution of investigational medicinal products, in order to provide a quick reference tool in a professional and academic context.

A review was made in the international literature to identify studies focusing on development and handling of investigational medicinal products, mainly through PubMed and Medline databases, although it has also resorted to guidelines, some books on the subject, and master and doctoral thesis. Only English language papers were selected. Whenever possible, it were selected the most recent articles.

During the development of IMP, solubility, as well as bioavailability assessment and bioequivalence studies, are the most challenging steps in the early phase of preparation of IMPs. Concerning the IMP production, the critical aspects, such as comparators, blinding and package, will determine the success of the entire clinical trial. When the IMP is fully prepared, it enters in the different clinical trials phases, with the aim of providing a range of information, such as efficacy and safety. This whole process must meet a series of requirements previously established, and adequate trained staff, in order to minimize the costs associated with the development of the IMP, as well as accelerate its market entry.

It is important to note some limitations in the review. Although there is a wide range of information on the preparation of investigational medicinal products, little information was found on the logistics. In a future work, it would be interesting to further explore the distribution of investigational medicinal products, addressing the main unsatisfactory results of audits, in a clinical trial environment, and find tools and procedures to prevent nonconformities.

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Abbreviations

(R)ADME	(Release), Absorption, Distribution, Metabolism, Excretion
API	Active Pharmaceutical Ingredient
BCS	Biophamaceutical Classification System
CD	Cyclodextrin
CE	Capillary Electrophoresis
CRC	Clinical Research Coordinator
CRF	Case Report Form
CRO	Contract Research Organization
CTM	Clinical Trial Material
EMA	European Medicines Agency
EP	European Pharmacopeia
GCP	Good Clinical Practices
GMP	Good Manufacturing Practices
HPLC	High-performance Liquid Chromatography
ICH	International Committee for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
JP	Japanese Pharmacopeia
NCE	New Chemical Entity
PCD	Positive Control Drug
PSD	Particle Size Distribution
R&D	Research and Development
SCF	Supercritical Fluid
SLN	Solid Lipid Nanoparticles
SOP	Standard Operating Procedures
USP	United States Pharmacopeia
UV	Ultraviolet Spectroscopy

CHAPTER 1

Introduction

Human curiosity has been the driver force for the advancement of science and medicine since these disciplines came into existence. In addition, human subjects have been used to substantiate the innovate theories involved in the pursuit of this knowledge.

The birth of the modern-day clinical trials is usually considered to be the publication by the United Kingdom Medical Research Council in 1948 of a trial for the treatment of pulmonary tuberculosis with streptomycin [1]. In any case, the comparative concept of assessing therapeutic efficacy has been known from the Old Testament [1, 2], but it was during the fourteenth to sixteenth centuries, in the Renaissance period, that the great development in many forms ranging from art to science took place [1].

The most famous historical example of a planned, prospective controlled, comparative, clinical trial is from the eighteenth-century: where Lind found oranges and lemons to be the most effective of six dietary treatments for scurvy on board ships [1, 2]. In the nineteenth-century, a clinician and pathologist, Pierre Charles Alexandre Louis, introduced the numerical aspect to comparing treatments [1], but, it was more than a century later when Bradford Hill used a formal randomization procedure for creating groups of cases that were "in all respects alike, except in the treatment" [1]. The randomization was a major contribution for the design of experimental studies and it refers to a scenario where patients are divided into groups and then the treatment for each group is randomly assigned [1]. However, beyond the randomization procedure, it also matters to highlight blinding, because today such procedures are considered the most important aspect of good, well-controlled clinical trials, unless the studies are to be open label, thus, not blinded. Blinding consist in a process of keeping hidden certain information about data, drugs or study procedures in order to help avoid bias. Most commonly this means keeping the treatment allocation hidden from the investigators and subjects (and often data management staff) taking part in, and managing a study [3].

Although clinical trials have an ancient history, it was in June 1964 that the World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles

to provide guidance to physicians and other participants in medical research involving human subjects. One of the principal topics of this declaration mentions that it is the duty of the physician to promote and safeguard the health of the subject. The physician's knowledge and conscience are dedicated to the fulfilment of this duty. Further, subjects will know exactly what to expected from a clinical study and from their participation, such as risks and benefits and that is why all have to provide informed consent before the clinical trial starts.

Nowadays, all clinical trials must be in compliance with the Declaration of Helsinki and there is a battery of regulatory legislation and guidelines that establishes all requirements and steps for the different clinical trials phases [4].

Since World War II, the clinical trial has evolved into a standard procedure in the evaluation of new drugs. Its features include the use of a control group of patients that do not receive the experimental treatment (the so called placebo group, when the study is to be placebo-controlled), the random allocation of patients to the different treatments, including the control group, and the use of blinded assessment. The clinical trial illustrates the desire of modern society to justify its medical choices on the basis of the objectivity inherent in statistical and quantitative data assessments, in support of an outcome for drug efficacy, and safety.

The major challenge of any drug discovery program is to bring together a cross section of talented, multidisciplinary scientists involved in evaluating and characterizing the pharmacological profile of a new drug entity [5]. The basic principles involve defining the mechanism of action, potency, efficacy, safety, toxicology, and metabolism of lead compound [5], in the first instance. The determination of the structural characteristics that may provide the desired biological activity, may be based on a rational drug design approach and classical structure-biological activity relationship [5]. However, nowadays, highly advanced computer technology allows a molecular approach based on computational of chemical entities, which may "fit" the active moiety of, for example, the selected and validated target enzyme, membrane receptor site, or a clearly characterized gene sequence [5]. In a following step, the compounds are evaluated by biochemists, cell biologists, pharmacologists, or biotechnologists using various *in vitro* screening assays (enzymes, membrane receptors, cellular organelles or isolated tissue preparations) to determine the intrinsic activity of the drug candidate [5]. In other words, the leads compounds are

optimized to generate the candidate. A drug candidate suitable for clinical testing is expected to bind selectively to the receptor site on the target, to elicit the desired functional response of the target molecule, and to have adequate bioavailability and biodistribution to elicit the desired responses in animals and humans; it must also pass formal toxicity evaluation in animals [6]. The path from lead to clinical drug candidate represents the most idiosyncratic segment of drug discovery and development [6]. Once the *in vitro* assessment has identified "active leads", the process goes on to *in vivo* testing of the drug candidate in appropriate animal models that best predicts the compound's ability to alter a particular disease target in humans [5]. In other words, the main goals of this process are to demonstrate the biological activity of the drug, to establish the therapeutic index by carrying out safety assessments in various species. Furthermore, analytical chemistry drives are the identification and characterization of potential metabolites that are produced by the parent compound [5], as well as potential by products and other sort of potential impurities. The various stages of preclinical studies involve a multidisciplinary expertise and the clinical pharmacologist group interacts with the preclinical scientists to remain informed of the findings of the drug candidate. All information is valuable to determine the first dose in Phase I clinical trials [5] and support pharmaceutical development to generate and provide information about formulation, manufacturing process, analytic and stability.

Preclinical studies of potential new medicines were relatively superficial until several disasters had occurred, in particular the thalidomide catastrophe in the 1960s, where exposure to this compound during early pregnancy resulted in limb deformities in developing embryos [7]. Further, early clinical studies were generally considered very safe until in March 2006, when several volunteers experienced severe immunological reactions after a first treated with TGN1412, a CD28 antibody, which, in hindsight, had been administered at a too high dose [7]. Today there are national and international regulations that require sponsors to provide information from a detailed package of preclinical studies, before they can apply for and start a clinical development program. Furthermore, although most investigators have heard of Good Clinical Practices (GCP), the interpretation of its meaning can vary enormously. In fact, the primary reason for the presence of a GCP code is the protection of human rights [4, 8]. Pressure to increase profits, cut drug development costs, and shorten regulatory approval times should not force the pharmaceutical industry to adopt a shameless "profit-over-people" [9].

After the preparation of the drug substance, pre-formulation and analytical studies for both the active and control substance, as well as the initial formulation, together with preliminary degradation studies and stability assessment are needed before the clinical program starts. All activities are lined up to support manufacture, control and release of the investigational medicinal product (IMP) for the clinical trials. According to the Portuguese Decree-Law number 176/2006 of 30 August and the European Directive [10] an IMP is defined as a "pharmaceutical form of an active substance or placebo, tested or used as a reference in a clinical trial, including drugs whose marketing there been authorized but are used or prepared on the dosage form or packaging, so different from the authorized form, or used for an unauthorized indication or for more information about the authorized form".

In clinical trials there may be added risk to participating subjects compared to patients treated with marketed products. So, it is crucial that IMP should be produced in accordance with the principles and the detailed guidelines of Good Manufacturing Practices (GMP) for Medicinal Products [11]. The application of GMP to the manufacturing of IMPs is intended "to ensure that trial subjects are not placed at risk, and that the results of clinical trials are unaffected by inadequate safety, quality or efficacy arising from unsatisfactory manufacture". Furthermore, the application of GMP should ensure "that there is consistency between batches of the same IMP used in the same or different clinical trials, and that changes during the development of an IMP are adequately documented and justified" [11].

The production of IMP involve complexity and various challenges in comparison to marketed products, due of the lack of fixed routines, variety of clinical trial designs, consequent packaging designs, and the need for randomization and blinding, and increased risk of product cross-contamination and mix up. Furthermore, there may be incomplete knowledge of the toxicity of the product and a lack of full process validation.

On top of this, the logistics associated with the preparation and distribution of IMP to the sites also differs from a standard route as used in a commercial product. Here, the randomization and the systems in place to control such distribution, based on a site need in a defined point in time, are crucial to guarantee that the right medication is at the site place in the right time.

Due to all of these challenges, moving from development to manufacture and to logistics, this project aim to make an overview about the development of an IMP, and the main

details that must be considered in the preparation, packaging, labeling and distribution of investigational medicinal products, in order to provide a quick reference tool in a professional and academic context. A review was made in the international literature to identify studies focusing on development and handling of IMP, mainly through PubMed and Medline databases, although it has also used guidelines, specialized books on the subject, and master and doctoral thesis. Only English language papers were selected, and whenever possible, the most recent articles were selected.

This master thesis presents the main studies needed to be carried out during drug pre-formulation, which will support the formulation stage. After formulation of the IMP in the convinient dosage form, preparation of IMP will be discussed in the next chapter, where concepts such as blinding, package and labeling will be of most interest. Subsequently, a brief discussion on the circuit of the IMP during the clinical trials will be reported.

CHAPTER 2

Development of Investigational Medicinal Products

2.1. Pre-formulation

The early stages of any new formulation include studies to collect basic information on the physical and chemical characteristics of the drug substance. These basic studies consist of pre-formulation work needed before product formulation begins.

Pre-formulation study is the key to developing a successful formulation. Physicochemical characterization of a new drug substance should be performed regardless of its intended use. Upon identification of a lead candidate the drug discovery group needs to invest resources in identifying and synthesizing appropriate forms of drug candidate or candidates. Salt of the drug substance can significantly affect various properties such as solubility, bioavailability, and stability of the drug substance. Once a drug candidate is identified, an understanding of various physicochemical properties such aqueous solubility, pH solubility, pKa (dissociation constant), and log P (partition coefficient) is essential prior to initiating any formulation development activities [12].

All IMP development is linked with chemical development, since this is where the development of a new chemical entity (NCE) starts. Furthermore, it is the mission of the chemists to ensure timely availability of NCE for use in other areas of development [13].

Some pharmaceutical companies have their own chemical development and production facilities, which greatly help, when in comparison with others that choose or are led to use outsourced manufacturing. In this last case, the transfer process becomes lengthy [13], but it would happen anyway in the development process unless the company has strong and industrial scale capabilities for manufacturing. When that is the case, a separate chemical development unit may be associated with the production plant [13].

Initial formulations for the majority of drugs are an injectable solution or dispersion for evaluation of basic pharmacology, bioavailability, pharmacokinetic and toxicology in

animals to confirm *in vitro* activity [14, 15]. However, approaches used during pre-clinical assessments may not be used when the drug is to be administered to humans, meaning that a more supported, detailed and extensive work need to be done when the scientists are working on developing a formulation to be used in a clinical trial. Here, all supportive information that can be gathered will help driving the development in the right path, avoiding steps back and forth and guiding not only formulation but also the establishment of the manufacturing process.

It is crucial to obtain fundamental physicochemical, mechanical and physical information on the NCE, to ensure that potential formulation strategies are consistent with the chemistry of the molecule [15] and feasible on a practical perspective.

There are several tests for a new drug molecule, but Wells consider two fundamental properties that are mandatory for a new compound: intrinsic solubility and dissociation constant (pKa) [15]. Others are, of course, of relevance and must also be determined, in support of the subsequent development steps. As well, and to allow the analytical control during development and the assessment of the formulation behavior, a simple analytical method must be developed [15]. The analytical pre-formulation includes:

- Identification of the drug: tests such as nuclear magnetic resonance, infra red spectroscopy, ultraviolet spectroscopy (UV), thin-layer chromatography, differential scanning calorimetry and optical rotation [15];
- Purity: moisture (water and solvents), inorganic solvents, heavy metals, organic impurities [15];
- Assay: titration (usually Karl Fischer titration is the method in analytical chemistry used, which determine trace amounts of water in a sample), UV, high-performance liquid chromatography (HPLC) [15];
- Quality: appearance, odour, solution colour, melting point and pH [15].

2.1.1. Solubility

Two important factors in the entire development process are drug solubility and bioavailability [12].

The time course of drug activity, drug blood levels, and levels in other body fluids will depend on a variety of factors including solubility and metabolism.

In whatever way drugs are presented to the body, they must usually be in a molecularly dispersed form (that is in solution) before they can be absorbed across biological membranes [16]. The solubilisation process will precede absorption unless the drug is administered as a solution, but even solutions may precipitate in the stomach contents, and the precipitated will then have to re-dissolve before being absorbed [16].

Drug solubility is an important parameter for both oral and intravenous administration [12]. Oral intake is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constrains, and flexibility in the design of dosage form [17]. However, the major challenge with design of oral dosage forms lies with the poor bioavailability of most of the drugs. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism, and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability [17].

There are many factors controlling the solubility of drugs in solution, such as the nature of the drug molecule and the crystalline form in which it exists, its hydrophobicity, its shape, its surface area, its ionization state, the influence of the medium pH and the importance of the pKa of the drug [16, 18].

It is very important to know the molecule's pKa in order to understand both physicochemical and bioavailability data.

Various techniques are available for solubility determination of drug substance. The oldest and the most commonly used solubility measurement technique was developed by Higuchi and Connors [12], that consists in adding the drug substance to a small volume of the solvent (usually 2-4 mL). The mixture is then allowed to equilibrate by shaking or rotating at ambient temperature for a period of at least 24 hours or until undissolved particles are observed at the bottom of the vial/tube [12].

Although most researchers like to carry out the saturation solubility study at ambient temperature, some choose to perform the study at 37ºC, which will provide accurate prediction of *in vivo* solubility when the drug is travelling through the gastro-intestinal tract [12, 15], which can assist the formulator in designing a robust formulation [12].

Nowadays, the NCEs under development are more intended for use as a solid dosage form and are also very poorly water soluble, fact that seems contradictory and, in fact, it is. Since some years ago, scientists started looking to this as an area of great development, since poorly water soluble drugs would theoretically, but in practice as well, originate bad absorptions and thus doubtful results in terms of pharmacokinetic parameters and *in vivo* interaction, e.g., with food. Also formulation challenges arose to bring scientists up to speed with the new information, techniques and know-how on solubility improvement.

It became clear that it would be crucial to enhance solubility and, consequently, bioavailability of these drugs. Curiously, but expectably, the major current challenges of the pharmaceutical industry are related to strategies for improvement the solubility of drugs [19], and so it is of greater interest to consider the different approaches used to improve it (table 1).

The techniques are chosen on the basis of certain aspects such as properties of the drug under consideration, nature of excipients to be selected, and nature of intended dosage form [17].

Table 1. Techniques for Solubility Enhancement of Poorly Soluble Water Drugs

Physical Modifications	Chemical Modifications	Miscellaneous Methods
Particle size distribution	Change of pH	Supercritical fluid process
Modification of crystal format	Complexation	Use of adjuvant
Solid dispersions	Salt formation	Hydrotrophy
Cryogenic techniques		

In summary, solubility of the active pharmaceutical ingredient (API) is a crucial attribute with respect to [20]:

- Selecting the dosage forms for clinical trials;
- Designing experiments to identify potential salt forms, cocrystal forms, polymorphic forms, solvates and hydrates;
- Aiding drug-manufacturing strategies.

2.1.1.1. Physical Modifications

2.1.1.1.1. Particle Size Distribution

A critical quality criterion for the solubility is the particle size distribution (PSD). Especially for poorly water soluble drugs the PSD is one of the characteristics that must be controlled, and usually leads to the establishment of a quality specification as part of the general quality control of the product. Techniques such as laser diffraction, using either Mie or Fraunhofer theory and optical microscopy (when the first cannot be used due to the shape of the particles or due to difficulties during method development) can be used to determine the PSD and can be complemented by a surface area determination [19].

Decreasing the particle size, leads to a surface area increase, resulting in a potentially increased dissolution rate, thus solubilisation, and, consequently, improved bioavailability. Reducing particle size is primarily done by milling or micronization, which consists in reducing particle size to a minimum in order to improve wettability, and, for consequence, bioavailability [19]. At this point, dissolution curves for different particle sizes can be made to verify what sizes have direct impact in dissolution and for which ranges the dissolution will be similar.

Apart from solubility, the particle size may also influence the uniformity of dosage of very potent drugs used in considerably small amounts and formulated as a solid dosage form [16]. Another example where particle size is of importance relates to direct absorption, for instance, in pulmonary delivery where only very fine particles are able to penetrate the alveolar regions of the respiratory tract [16]. However, if the particle size is reduced too

far, particles may be exhaled and not deposit [16], resulting in less absorption extent and thus potential lack of efficacy due to dose loss.

Particle size also has important effects on the bulk properties of a powder flow during manufacturing processing when large quantities of material are handled. It is important that pharmaceutical powders are able to flow freely into storage containers or hoppers of tablet and capsule-filling equipment so that a uniform packing of the particles and hence a uniform tablet or capsule weight is achieved [16].

Despite being a fast method to improve solubility, micronization also has several disadvantages related to the limited opportunity of control important characteristics of the final particle such as size, shape, morphology, surface properties and electrostatic charges [21].

It becomes important to refer to the nanosuspension as a particle size reduction technique, since the major problems of the other techniques is lack of universal applicability to all drugs [22]. Over the last decades, nanoparticle engineering has been developed and reported for pharmaceutical applications [22, 23], enhancing solubility of drugs that are poorly soluble in water [24]. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster.

2.1.1.1.2. Modification of Crystal Format

Often for drugs with very low aqueous solubility, the achieved increase in dissolution rate is insufficient to provide adequate enhancement of bioavailability. The potential for increased Van der Waals interactions and electrostatic attraction between ultrafine particles can also act to reduce the effective surface area for dissolution and therefore limit improvements in bioavailability [25]. Crystal engineering approaches, which can potentially be applied to a wide range of crystalline materials, offer an alternative method for improving the solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. The ability to engineer materials with suitable dissolution characteristics, maintaining suitable physical and chemical stability, provides a strong driver for the use of new and existing crystal engineering approaches to drug delivery system design [25].

Crystallization is concerned with the evolution from solution or melt of the crystalline state. Within this area, key issues include the formation of crystal nuclei, the influence of crystallization conditions, and the overlap between the concepts of the growth unit, and an understanding of how the overall shape of a crystal evolves [25].

Once nucleation has been achieved, crystal growth leads to the evolution of embryonic crystals into a crystal form of defined size and shape. The key drivers with regard to the shape of the growing crystal are related to the crystal lattice of the molecular solids and the effects of the choice of solvent and additives on the process of crystal growth. As such, crystal growth is a layer-by-layer process, with the evolution of the layers being defined by the crystal packing of the unit cell. The unit cell in turn describes the critical elements of how a specific molecular species has assembled in a crystalline state in three dimensions [25].

The crystal morphology depends on the conditions of crystallization and may affect the syringeability of suspensions of the drug, its ease of compression into tablets and its flow properties [16]. The crystal formats can be modified generally by adding surfactants to the solvent used for crystallization [16].

Knowing that the amorphous form of a chemical substance is usually more soluble than the crystalline form, different extents of drug absorption may result from here with consequent differences in the degree of pharmacological activity. Most of the developed drugs are present in a crystalline form and not in its amorphous form. This drives scientists in looking for other alternative approaches, which also includes changing the substance to its amorphous form (topic 2.1.1.1.3.).

However, crystalline forms of drugs may be used because of greater stability than corresponding amorphous forms [21]. Also, the various polymorphic forms can differ in drug action (pharmaceutically and therapeutically) because they differ in many physical properties [21].

By manipulating the crystallization conditions it is possible to prepare crystals with different packaging arrangement: such crystals are called polymorphs [17]. As a result, the majority of drugs can crystallize into several polymorphs. Each polymorph has a different energy, showing different physicochemical properties, such as melting point, density, solubility, dissolution rate and stability [26]. Generally, the solubility of metastable

polymorphs is kinetically higher than that of a thermodynamically more stable polymorph [17, 26].

2.1.1.1.3. Solid Dispersions

Over the past few years, interest has been shown in solid solutions of drugs in attempts to change the biopharmaceutical properties of drugs that are poorly soluble or difficult to wet. The main goal is usually to provide a system in which the crystallinity of the drug is changed to increase its solubility and dissolution rate, and to surround the drug intimately with water soluble material [16]. This approach has a tremendous potential and the literature reviews of the past four decades of research suggest that there is an increasing interest in using it [21].

Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption, and therapeutic efficacy of drugs in a specific dosage form [17]. The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug [17].

Vasconcelos [19] described three solid dispersions generations. The first generation was prepared using crystalline carriers, such as urea and sugars, that produced higher bioavailability, but it have the disadvantage of forming crystalline solid dispersions, which were thermodynamically more stable and did not release the drug as quickly as the amorphous ones [19]. Then, a second generation of solid dispersions appeared, which contained amorphous carriers instead of crystalline. Polymeric carriers have been the most successful for solid dispersions, because they are able to originate amorphous solid dispersions [19]. In amorphous solid solutions, drug and carrier are totally miscible and soluble, originating a homogeneous molecular interaction between them, resulting in a really true solution [19]. On the other hand, drugs with a high melting point and/or limited carrier solubility are candidates for producing an amorphous solid suspension [19]. Recently, third generation solid dispersions appeared which contains a carrier or a mixture of amorphous polymers and surfactants. This last generation is intended to achieve the highest degree of bioavailability for poorly soluble drugs and to stabilize the solid dispersion, avoiding drug recrystallization [19]. The use of surfactants as carriers was

shown to be effective in originating high polymorphic purity and enhanced *in vivo* bioavailability. In addition, the use of surfactants may help to prevent precipitation and/or protect a fine crystalline precipitate from agglomeration into much larger hydrophobic particles [19].

Solid dispersions are easier to produce and the application is now wider than the salt formation. Furthermore, solid dispersions are more acceptable to patients, since they give rise to solid oral dosage forms instead of liquid ones [19].

In summary, it is possible to conclude that crystalline substances are more stable than amorphous substances, but the amorphous ones are more soluble. The increase in dissolution rate for solid dispersion can be attributed to a number of factors, such as reduced particle size or reduced agglomeration [21]; a possible solubilisation effect by the carrier, which may operate in the diffusion layer [16]; improved wettability in the intimate drug-carrier mixture [16], transferring the drug from a crystalline to amorphous state [21], more soluble, among others factors.

2.1.1.1.4. Cryogenic Techniques

Cryogenic techniques have been developed to enhance the dissolution rate of drugs by creating, at very low temperature conditions, nanostrutured amorphous drugs particles with high degree of porosity [17].

Cryogenic inventions can be defined by the type of injection device (capillary, rotary, pneumatic and ultrasonic nozzle), location of nozzle (above or under the liquid level), and the composition of cryogenic liquid (hydrofluoroalkanes, N_2, Ar, O_2 and organic solvents) [17].

After cryogenic processing, dry powder can be obtained by various drying processes like spray freeze drying, atmospheric freeze drying, vacuum freeze drying, and lyophilisation [27].

2.1.1.2. Chemical Modifications

2.1.1.2.1. Change of pH

Another technique is the adjustment of the pH of the solvent to enhance solubility. However, for many drug substances pH adjustment is not an effective means of improving solubility [28].

The effect of pH on solubility is critical in the formulation of liquid dosage forms, from oral and topical solutions to intravenous solutions. The solubility of a weak acid or base is often pH dependent. However, weak acidic or basic drugs may require extremes in pH that are outside accepted stability ranges. In many cases, it is desirable to use co-solvents or other techniques such as complexation, micronization, or solid dispersion to improve aqueous solubility [28].

Modifications of pH in solid dosage forms is considered to be an alternative option for an ionizable drug in order to improve the solubility and dissolution rate. The incorporation of pH modifiers in the dosage form can alter the micro-environmental pH. Micro-environment is a term used to represent a microscopic layer surrounding a solid particle in which the solid forms a saturated solution of adsorbed water [29]. The micro-environmental pH would affect the performance of the solid dosage form, such as the chemical stability of the drug substance and the dissolution profile [30]. There have been several studies demonstrating the pH-independent release of basic drugs form controlled release dosage forms by using pH modification technologies [26].

To obtain the complete dissolution of the drug from dosage form, the pH modifier may need to coexist with the drug particles in tablet or granule until the containing drug is completely dissolved. Accordingly, the excipients and manufacturing methods would affect the dissolution performance of the drug from the pH-modified solid dosage forms. The estimation of the micro-environmental pH in the dosage form is thought to be helpful in designing pH-modified dosage forms [26].

2.1.1.2.2. Complexation

Among all the solubility improvement techniques, inclusion complex formation technique has been employed more precisely to enhance the aqueous solubility, dissolution rate and, consequently, bioavailability of poorly water soluble drugs [17].

Inclusion complexes are formed by insertion of the nonpolar molecule or the nonpolar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host). The most commonly used host molecules are cyclodextrins (CD) [17].

CDs occur naturally and are obtained through the enzymatic reaction on the starch [16] [31]. CDs are cyclic oligosaccharides built up from glucopyranose units linked by α-1,4 bonds, thus forming a torus-like macro-ring [16, 31]. They are characterized as crystalline, water-soluble, homogenous, and non-hygroscopic substances, and they may modify important properties of the drugs through their inclusion complex formation ability [17, 31].

CD molecules surface gives them water-soluble properties, but the hydrophobic cavity creates a micro-environment for non-polar molecules. Based on the intrinsic properties of the molecule, such as its structure, drug-CD complexes can be generated in 1:1 or 1:2 proportions as illustrated in figure 1.

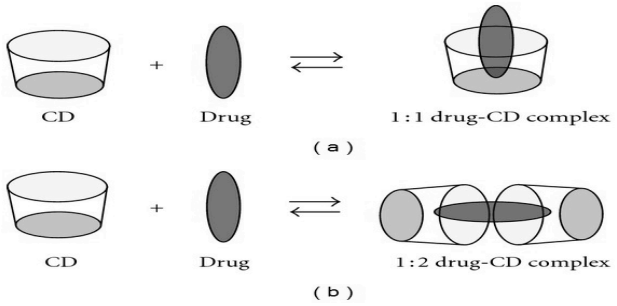

Figure 1. 1:1 and 1:2 Drug-Cyclodextrin Complexes. Adapted from Davis and Brewster (2004) [32]

2.1.1.2.4. Salt Formation

The majority of NCE do not have water solubility since they are organic molecules of low molecular weight, generally, weak acids or weak bases, and therefore salts may be required [14]. One of the major improvements in solubility can be achieved by forming a salt, and this is the most preferred approach for the development of liquid formulations for parenteral administration [18].

The dissociation constant (pKa) of the parent molecule must be known prior to the selection of the salt and, generally, this selection is determined from a shortened list of potential pharmaceutical acceptable counter ions [33].

In case of strong acids or strong bases, salts prepared are freely soluble but very hygroscopic, leading to instability in tablet or capsule formulations. Therefore, weak acid or base solutions are preferred even though they have less solubility, but are also less hygroscopic.

During the salt selection process, attempts are generally made to minimize the potential for multiple forms of drug. So, consideration for the proper crystal modification to be developed for clinical evaluation should occur early in the development [33]. In fact, this is one of the major concerns: some drugs can crystallize in more than one polymorphic form characterized by differences of packaging in the crystal lattice or in the orientation or conformation of the molecules at the lattice sites [16]. Polymorphs of the same drug may have different melting points and solubility, and usually exist in different habits [16]. Polymorphs may cause problems in the formulation, analysis and, especially, bioavailability of drugs. The development of specific polymorphs is in disuse, and the trend is the drug modifications using new drug delivery systems, consisting in the modification of the API's structure to originate intrinsic characteristics that improves solubility, and, for consequence, keeping the stability of the drug.

Furthermore, processes involved in manufacturing, such as grinding, granulation, compression, or mixing, can also affect and originate changes in crystal form [33]. To avoid these problems, screening for crystal modifications should be completed before initiation of preclinical safety assessment studies that will support clinical development [33]. This evaluation can be effected, for example, altering recrystallization solvents and

temperatures, exposing purified drug substance to varying humidity conditions, and performing compaction stressing of the drug substance [33].

Salts are usually prepared from organic solvents and they are destined to encounter aqueous environment (water, humidity) during dosage form development, and at the time of dissolution in gastrointestinal fluid, in case of an orally administered tablet or capsule. This can be a major issue and its use may be limited, since a perfectly good salt isolated from an organic solvent may not behave well in an aqueous environment due to low solubility, conversion to free acid or base forms and poor stability [18].

2.1.1.3. Miscellaneous Methods

2.1.1.3.1. Supercritical Fluid Process

Another novel nanosizing and solubilisation technology, whose application has increased in recent years, is particle size reduction via supercritical fluid (SCF) processes. According to Savjani *et al.* [17], SCF are fluids whose temperature and pressure are greater than its critical temperature (Tc) and critical pressure (Tp), allowing it to assume the properties of both a liquid and a gas. At near-critical temperatures, SCFs, are highly compressible allowing moderate changes in pressure to greatly alter the density and mass transport characteristics of the fluid that largely determine its solvent power [17, 34]. Thus, reducing the particle size, solubility increases.

2.1.1.3.2. Use of Adjuvants

The use of surfactants improves the dissolution performance of poorly soluble drug products, and is probably the basic, primary, and oldest method applied. The surfactants reduce surface tension and improve the dissolution of lipophilic drugs in aqueous medium, but they are also used to stabilize drug suspensions [17].

When the concentration of surfactants exceeds their critical micelle concentration (which is in the range of 0.05-0.10% for most surfactants), micelle formation occurs which entrap the drugs within the micelles. This is known as micellization and generally results in enhanced solubility of poorly soluble drugs [17, 35].
Surfactant also improves wetting of solids and increases the rate of disintegration of solid into finer particles. Furthermore, surfactants are often used to stabilize microemulsions and suspensions into which drugs are dissolved [35].

2.1.1.3.3. Hydrotrophy

Hydrotrophy is a solubilisation process, which consists in an addition of a large amount of second solute, the hydrotropic agent, resulting in an increase in the aqueous solubility of first solute. So, the hydrotrophy designates the water solubility increase due to the presence of large amount of additives. The mechanism by which it improves solubility is more closely related to complexation, involving a weak interaction between the hydrotropic agents, like sodium benzoate, sodium acetate, sodium alginate, urea, and the poorly soluble drugs [17].

2.1.2. Partition Coefficient

Partition coefficient (Log P) is a key property in determining hydrophilic or lipophilic characteristics of the NCE [12], and it is defined as the ratio of concentrations of the substance in oil and in water. With the advent of the high-throughput screening, a technique that allows the screening of thousands of new compounds in a very short period of time, several molecules identified in very early stage are lipophilic, with large molecular weight and, therefore, cause serious challenges due to their solubility and permeability limitations [12, 36].

Partition coefficient is also a good indicator of diffusion or permeation across the cell membrane, which is the rate-limiting step in the drug transport process [12].

2.1.3. Dissociation Constant (pKa)

Dissociation refers a process in which the compound is separated into smaller ions or particles. The portion of the drug dissociated can be represented by the dissociation constant (pKa), also known as ionization constant. Ionization plays an important role in determining the permeability of the drug molecule across various physicochemical membranes [12].

Dissociation constant, or pKa, is usually determined by potentiometric titration. However, for this method to be effective the drug must be at least slightly soluble in aqueous media [12, 28].

Nowadays, high-throughput techniques are available for measuring ionization constant, such as the most recent: capillary electrophoresis (CE) [37]. The CE technique offers advantages over traditional techniques because uses much less sample than other techniques available [37]. Moreover, because it is based on an analytical separation technique, several substances can be measured simultaneously. The basis for determination of dissociation constants by CE is measurements of the migration time of the analyte in electrolytes with a well-defined pH. In practice, the mobility is calculated from the migration time for the analyte as well as for the electro-osmotic flow [37]. Several studies have demonstrated the use of CE applicated to pKa, and some authors have suggested the use of CE for unstable compounds in aqueous solutions [37]. A challenge can be raised when analyzing labile compounds, but Ornskov *et al.* showed that pKa can be determined with high precision and accuracy also for labile drug compounds using CE [37].

2.1.4. Membrane Permeability

Due to poor physicochemical properties of drug substance, the ultimate aim of the drug development process is to increase the cell-membrane permeability, and to achieve significant intestinal absorption. Also, regardless of the route of administration, cell membrane permeation is required for a drug molecule to reach the general circulation [12].
Modern pre-formulation studies include an early assessment of passage of drug molecules across biologic membranes, and such permeability studies play a key role in determining the formulation strategy [12, 28].
Data obtained from the basic physicochemical studies, specially, pKa, solubility, and dissolution rate, provide an indirect indication of absorption [28]. However, a reliable screening method is required to assess the permeability of the drug. Various *in vitro* models are available for the prediction of membrane permeability. The major advantage of artificial membrane permeability over biological preparations are reproducibility, whereas, the major limitations are lack of enzymes, transporters, and paracellular pathways [38].

2.1.5. Chemical Stability

Many drugs are susceptible to some form of chemical decomposition when formulated in their liquid or even solid dosage forms. Figure 2 shows some of the factors that influence in drug stability according to pharmaceutical dosage forms.
Chemical degradation of the active drug can lead to a substantial lowering of the quantity of the active moiety in the dosage form. Furthermore, the low therapeutic efficacy can also result as a consequence of physical and chemical changes in the excipients present in the dosage form [16]. Although chemical degradation of the API may not be extensive, a toxic product may be formed in the decomposition process, causing undesirable effects [16].

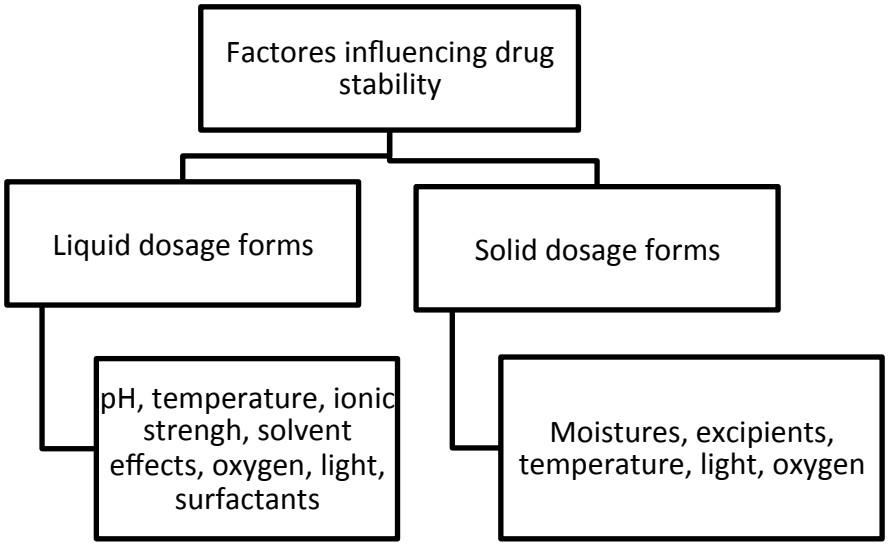

Figure 2. Examples of Factors Influencing Drug Stability of Liquid and Solid Dosage Forms

The chemical stability studies conducted on solid dosage forms under a range of different storage conditions and over different periods of time, aim to evaluate the chemical degradation at a range of various temperatures (depending upon stability), variable levels of relative humidity up to 90% [14, 39] and exposure to artificial or natural light (photostability), and in the presence of oxygen [40]. These studies can also be conducted on aqueous solutions at various pHs and temperatures and in a variety of solvents [14].

The primary cause of degradation of APIs in aqueous systems is hydrolysis[1] and the most susceptible drugs are those containing ester, amide, lactone, lactam, imide or carbamate groups. The inclusion into the formulation of non-aqueous solvents may, in some cases, reduce hydrolysis by alteration of the dielectric constant [16].

The second most common cause of drug breakdown is oxidation[2]. Oxidative degradation represents a problem with drugs possessing carbon-carbon double bonds such as steroids, polyinsaturated fatty acids and polyene antibiotics [16].

[1] Hydrolytic breakdown is catalysed by hydrogen and/or hydroxyl ions and, consequently, formulating the product at the pH of maximum stability may reduce the extent of hydrolysis.

[2] Oxidative degradation can occur by autoxidation, in which the reaction is uncatalysed and proceeds quite slowly under the influence of molecular oxygen, or may involve chain processes consisting of three concurrent reactions: initiation; propagation and termination.

Photochemical decomposition has been also considered an important cause of degradation, which can be a serious problem that can cause discoloration and loss of therapeutic activity of the drug. Photodecomposition might occur, not only during storage, but also during the handling of the product. Formulations containing light-sensitive drugs have to be stored in appropriate opaque or coloured containers, which either remove the ultraviolet components of light [16] or avoid light to pass through.

The forced degradation studies, performed during development to support subsequent steps in drug development, indicates how the drug should potentially degrade and also provide information for manufacturing process and packaging requirements. For example, if the drug is rapidly oxidized, the formulator can add certain excipients or change the manufacturing or packaging processes to minimize this degradation pathway [40]. The methods used along pre-formulation studies, needs to be adjusted to resolve new degradation processes involving products or excipient-related components [33].

Stability studies will give the feasibility of various types of formulation and packaging, being fundamental in following steps of drug development [14].

2.1.6. Excipient Compatibility

An excipient is defined as a material that is deliberately incorporated into the formulation to aid in a part of the process, increase the process feasibility or, simply, to improve the aspect or taste of the pharmaceutical form. Thus, excipients can improve tablet integrity, dissolution, bioavailability, or taste, amongst other characteristics. Moreover, excipients can also assist in modifying or permitting drug delivery [2] and can also improve stability of the API [40].

A successful formulation depends on the careful selection of excipients, which should not interact with the drug substance, or any other excipient.

Before formulation starts, studies to choose the excipients should be performed, with the aim to study drug-excipient and excipient-excipient mixtures, using differential scanning calorimetry at typical processing temperatures, which requires small samples of API. No interaction indicates potential stability [14], but, additionally, another studies are useful for

early identification of any potential excipient-excipient interactions that can confuse analyses [33, 40]. There are several sources of information on excipients used in approved drug products [33, 40].

In summary, pre-formulation testing provides a basic dossier on the compound and plays an important role in identifying possible problems and suitable approaches to formulation [14]. However, some pre-formulation studies may need to be repeated during the development process when changes to the API or its manufacturing procedure occurs [40]. During all the formulation development it is important to be aware that some excipients may alter the pharmacokinetics of a given drug [2].

2.2. Formulation

Only correct formulation can turn a new API into a safe and ready-to-use drug, that can be dosed as required. Formulation scientists work to ensure that the body can absorb the substance and the therapeutic dose reaches the targeted organ. Not every drug is suitable for ingestion as a standard tablet, and special demands on the form of administration – injection into the eye, products for inhalation, or orodispersible tablets that dissolve quickly in the patient's mouth “without water” – regularly create new challenges for formulation scientists.

Formulation can be defined as an experimental stage in drug development to set a physical system that can be used, keeping in mind process feasibility, quality of the product and stability. The transformation of a drug into a medicinal product is a complex process that is controlled by a range of competing factors [14], such as patient acceptance and specific requirements of the API.

It is important to consider certain principles of the formulation, especially with focus on clinical trials environment [39]:

- A suitable formulation permits the conduct of clinical trials, mainly because it strongly influence patient acceptability;
- Formulations constrain clinical trial design. For example, bioavailability must be reconciled with toxicology coverage, well-

matched placebos may or may not be available, and special procedures may be required;

- Product storage and stability (or lack thereof) can bias the results of clinical trials.

The choice of formulation will significantly impact other parameters, such as efficacy, safety, manufacturing, and, obviously, the cost. Pharmaceutical scientists work closely with discovery scientists, clinical operations staff, manufacturing scientists and regulatory managers to devise the best possible formulation for patients at a reasonable cost for the company [40]. It should be noted, however, that formulation costs tend to decrease from development to commercial due to scale savings. The batch scales used throughout development and to commercialization of a product present differences, sometimes significant, thus impacting the global costs on an unit basis.

Many characteristics studied in early development such as solubility, stability, safety, potency, half-life, and molecular size will strongly influence the product profile [40] and must be considered when formulating.

Further, it s important to keep in mind that a medicine is not just an active compound, but also includes excipients, degradants of the active compound, degradants of the impurities and those from excipients [39], that must be considered during formulation, so that all knowledge must support analytical development for future control of the developed product. Impurities and degradants can have their own toxic potential, so is important to control impurity content in early development. But, most of times, the structures of these impurities and degradants may not be well characterized, being typically common both bulk drug and finished product to become more refined as clinical development proceeds [39]. It is common practice, and also a safe measure, to use "*less pure"* bulk drug for toxicology studies, in order to restrain any new toxicology problems developing later during clinical research [39]. Then, as the clinical program develops, batches tend to be more and more pure, most of the times due to changes and adaptations in the synthetic routes of the API or in the manufacturing process itself.

All data from pre-formulation studies and preclinical studies are crucial to choose a particular route of administration [41]. Since, the formulation must also be suitable for rapid and economical manufacture [14], commonly, pharmaceutical companies prefer to develop an oral dosage form, in most cases a solid one. Dosage forms associated with oral

administration are relatively cheap to manufacture, are simpler for patients to handle, and are widely accepted by the public [33]. Because of these facts it becomes critical to evaluate the oral bioavailability of candidate compounds in the early stages of development.

Bioavailability is a term that describes the pharmacokinetic "speed" and extent to which an active compound or its active moiety is released from a drug product, absorbed by the human body and becomes available at the site of action. The assessment of bioavailability is performed based on pharmacokinetic parameters calculated from the plasma concentration profiles of the drug over time. The bioavailability studies allow not only to assess the quality of medicine, based on its pharmacokinetic profile, but also to evaluate drug interactions and the influence of various physiological and pathological factors (age, diet disease, especially renal and hepatic function, etc.) in the drug absorption.

Understanding oral bioavailability of a compound provides a significant insight of the strategy of the formulation development [33]. As already discussed, some formulation can significantly improve bioavailability, predominantly if the reason for the poor bioavailability is related to the drug's solubility [40]. If preclinical studies indicate an acceptable bioavailability of the molecule, then the best approach is to dose orally using a simple dosage form, such as solutions or encapsulated drug substance [33].

A classification system was developed to relate drug intestinal absorption to the physical properties of drugs (such solubility), in which drugs are classified in four classes (table 2).

Table 2. The four Biophamaceutical Classification System (BCS). Adapted from Florence and Attwood (2011) [42]

The four Biophamaceutical Classification System (BCS)	
Class I: High solubility, high permeability	Class II: Low solubility, high permeability
Class III: High solubility, low permeability	Class IV: Low solubility, low permeability

2.2.1. Bioavailability and Bioequivalence

As mention above, bioavailability is a term that describes the pharmacokinetic speed and extent to which an active compound, or its active moiety, is absorbed from a drug product and becomes available at the site of action. Considering the various stages through which a drug substance is intended to be absorbed in the body, a series of phenomena are referred to by the acronym (R)ADME, which means: (release), absorption, distribution, metabolism and excretion.

The "release" is referred as the stage that most affect the bioavailability [43]. There are many attempts to overcome the obstacles that block a proper absorption, being common the use of pro-drugs that cross better semi-permeable membranes, than the drug itself [43]. Thus, bioavailability between 80 or 100 per cent is not an easy task, especially considering a whole load of excipients and adjuvants, which is necessary to add.

Both the rate and extent of drug absorption can be substantially modified by formulation and processing variables: if there is any significant change in the formulation or process during the clinical trials development period, the questions of bioequivalence needs to be addressed [44].

The purpose of a development bioequivalence study is to build a bridge between a product used in clinical trials with a version that will actually be marketed [44] or eventually used in a different phase of the clinical development program. Thus, whenever a new dosage form of the drug is developed, it must demonstrate that it is bioequivalent with respect to the original form to establish the safety and efficacy of the drug. Also, when a formulation has been used in clinical trials and another separate formulation is selected for the product to be marketed, a bioequivalence test will be need [44].

2.2.1.1. Factors Affecting Bioavailability

Bioavailability of a drug depends upon pharmaceutical factors related to physicochemical properties of the drug and characteristics of the dosage form, pathophysiology of the disease, and route of administration [45]. Table 3 shows the major factors that affect bioavailability.

Two major problems can be identified that result in poor bioavailability of an orally administered drug [45]:

- Degradation of the drug into inactive form;
- Interaction with one or more components of the dosage form or those present in the gastro-intestinal tract to form a complex that is poorly soluble or is unabsorbable.

Table 3. Factors Affecting Bioavailability

Factors affecting Bioavailability		
Physicochemical Properties of the Drug Substance	**Physiological Factors**	**Physicochemical Interactions in Dosage Form**
Polymorphism	Gastrointestinal motility	Drug-excipient interactions
Salt Form	Gastric Emptying	Excipient-excipient interactions
Pro-drug	Intestinal Transit Rate	Effect of excipients on physiological processes
	Food and pH Effect	Modification of Biorelevant Drug Product Properties by excipients
	Window of Absorption	
	Variability in Metabolizing Enzymes and Efflux Transporters	

Concerning physiological factors, gastric emptying provides greatest influence on the rate of oral drug absorption, since an orally administered dosage form encounters the stomach first. Also, gastric muscles exert mechanical pressure on the dosage form. Gastro-intestinal transit times can influence oral drug bioavailability through a multitude of mechanisms, such:

- *Gastric empty*: for the drugs administered as solid particles or tablets, rapid gastric emptying can lead to incomplete drug dissolution in the stomach, which can further reduce the extent of drug absorption [45].
- *Intestinal Transit Rate*: general increases in intestinal motility can increase the rate of drug transport from one intestinal segment to the next. Several factors can affect gastro-intestinal motility, such as pharmacological effect of the drug itself [45].
- *Food and pH Effect*: food intake can affect drug absorption either by directly interacting with the dosage form or by affecting gastro-intestinal physiological parameters relevant to drug absorption. Food also influences gastric pH: while the normal gastric pH is 1-3 in fasted state, the fed state gastric pH in humans can be 4.3-5.4 [46]. The effect of gastric pH on oral drug absorption can be most predominant for weakly basic compounds that have high solubility at acidic pH in the stomach and low solubility at the basic pH in the intestines. The rate and extent of oral bioavailability of these drugs in humans is dependent on their rapid dissolution from an oral solid dosage form in the acidic stomach [45].
- *Absorption window*: some drugs are only soluble at a particular pH or they are absorbed using a specific mechanism. These drugs can only be absorbed in specific segments of the gastro-intestinal tract. Those particular segments are named "absorption windows" [45].
- *Variability in metabolizing enzymes and efflux transporters*: several drugs are substrates of the drug metabolizing enzymes in the gastro-intestinal tract, such as cytochrome P450 (CYP) enzymes in the intestinal mucosa, and efflux transporters, such as the P-glycoprotein (P-gp) family of transporters. Oral absorption of drugs that are substrates of the efflux transporters and metabolizing enzymes is understandably affected by the inter-individual expression level and intra-individual distribution of these proteins in the gastro-intestinal tract. For example, P-gp transport has been linked to the low and variable oral bioavailability of several compounds [47]. In addition, drugs whose absorption is affected by transporters and metabolizing enzymes can also be sensitive to certain food effects [48].

Excipients can also affect drug bioavailability through physicochemical interactions in the dosage form that can affect drug absorption. Such interactions could be drug-excipient or excipient-excipient interactions [45]. The most common interactions regarding excipients are drug-excipient interactions [49]. Drug-excipient interactions can be a result of physical (polymorphism, crystallization), chemical (oxidation, hydrolysis) or biopharmaceutical interactions. Excipients can initiate, propagate or participate in physical or chemical interaction with drugs, affecting the therapeutic efficacy of the drugs. Thus, interactions in solid dosage forms between its components and of its components with the physiological processes can affect the bioavailability of drugs. Understanding these phenomena is crucial to avoid their undesired consequences [45].

Since excipients can interact directly with physiological processes, such as pH of gastro-intestinal fluids in the immediate vicinity of the dosage form, gastro-intestinal transit time, effective membrane permeability, drug degradation in the gastro-intestinal fluids and drug metabolism and efflux during absorption, they can alter the rate and extent of drug absorption [45]. The effect of excipients on gastro-intestinal motility is dependent on their concentration, and can influence drug absorption. This effect can be avoid or minimized by lowering its concentration in the formulation [45].

2.2.2. Dissolution Testing

Laboratory tests to measure physical properties *in vitro* can give insights into the quality of the product. One of the common tests applied to oral dosage forms, in particular, is the measurement of drug rate of release by using dissolution test.

In the pharmaceutical environment, dissolution testing is an important tool in drug development and quality control. Although initially developed for immediate release solid oral dosage forms, and then for modified release solid oral dosage forms, the application of dissolution testing has expanded to a variety of another dosage forms. As these formulations have become more prevalent due to complexities of drug delivery, there has been an increased development of modified testing methods to characterize the *in vitro* release of these dosage forms [50].

For orally administered immediate release solid drug products, it is common to refer to the test as a "dissolution" test, since the intention is to dissolve the drug in the test medium. However, for non-oral dosage forms, such as topical and transdermal delivery systems, suppositories, and others, the test is referred to preferably as a "drug release" or "*in vitro* release" test procedure. As special dosage forms exhibit significant differences in formulation design, which in turn leads to very different physicochemical and release characteristics, it is not possible to devise a single test system to study the drug release properties. Rather, different apparatus, procedures, and techniques are employed on a case-by-case basis, and the method may be specific to the dosage form category, formulation type, or even to a particular individual product [50].

Since most of the investigational medicinal products are intended for solid oral dosage form, this topic will give more importance to that dosage forms.

First, it is necessary to define dissolution and dissolution testing. Dissolution is the process of extracting the drug substance out of it dosage form into solution within the gastrointestinal tract [51]. As described before, absorption is the process of transporting the drug substance from the gastrointestinal tract into the systemic circulation. So, dissolution testing is an *in vitro* method designed to demonstrate how efficiently an active drug substance is extracted out of a solid oral dosage form [51, 52]. Figure 3 shows, schematically, the relationship between dissolution test and absorption of a drug.

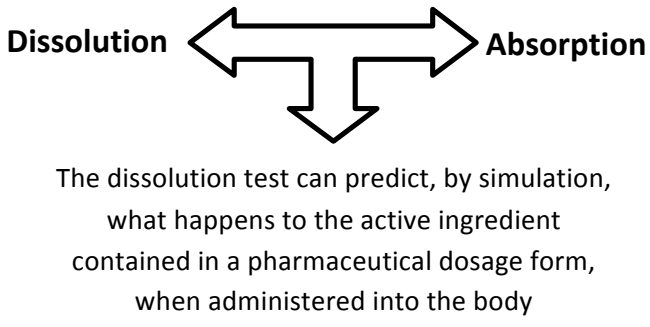

Figure 3. Relationship between Dissolution Test and Absorption

Dissolution provides very useful information on the performance capabilities of solid oral dosage forms that may be used to assist in the selection of suitable formulations. Also, it serves as a quality control test in support of routine manufacture to establish lot-to-lot

performance consistencies. In fact, this test method is considered so useful that it is a standard method published by the United States Pharmacopeia (USP), the European Pharmacopoeia (EP), and the Japanese Pharmacopoeia (JP) [51].

Dissolution testing is of great relevance, especially when [52]:

- The drug has low aqueous solubility;
- There is evidence that particle size may affect bioavailability;
- The physical forms of drug (polymorphs, solvates or complexes) have different solubility and dissolution characteristics;
- Specific excipients alter dissolution or absorption;
- There is evidence that tablet or capsule coating may interfere with disintegration or dissolution.

The main applications for testing the dissolution are [51]:

- To study the release kinetics;
- To develop and optimize a formulation;
- To control manufacturing;
- To compare the performance of various dosage forms or various formulations;
- To support scale-up and post-approval changes for commercial immediate release and modified release product – the dissolution rate and equivalence data required is dependent on the magnitude of the changes;
- For development of immediate release of the generic products – demonstration of equivalence of the potential generic version with the innovator product;
- For new products - clinical trials are often conducted using an established commercial product as a comparator. Because it is important that neither the investigators nor clinical trial subjects are able to distinguish among the product under investigation, the clinical comparator, the test, and any placebo (blinding), the various products are made to look alike, meaning are blinded [51]. Achieving this with a commercial product usually involves some form of manipulation such as over-encapsulation and then addition of a backfill. Recognizing that such manipulation could affect the bioavailability of the comparator, the European

Medicines Agency (EMA), in its "*Guideline on the Requirements to the Chemical and Pharmaceutical Quality Documentation Concerning Investigational Medicinal Products in Clinical Trials*", requires dissolution testing to be conducted to demonstrate that manipulations of comparator products have not compromised their performance [53].

2.2.2.1. Types of Dissolution Apparatus

Any dissolution test method consists in immersing the dosage unit in a suitable medium, which is kept in motion at a constant speed and requires the determination of the rate at which the drug substance is extracted from the dosage unit and dissolves in the medium [51, 52]. Results of this type of determination are dependent on system hydrodynamics, which, in turn, depend on apparatus details. The various compendia provide strict details of all dissolution apparatus dimensions. Fortunately, the chapters on dissolution published in USP, EP, and JP are harmonized [51], and single dosage units are tested in a number of vessels or cells (usually six).

For orally administered immediate and delayed release dosage forms, including tablets, capsules and suspensions, USP Apparatus 1 (basket) or 2 (paddle) is recommended (figures 4 and 5) [52], but generally apparatus 2 (rotating paddle) is the first choice for immediate release, due to ease of use, reproducibility, hydrodynamics, and general acceptance [51].

For extended release dosage forms, designed to deliver the drug to site absorption at a controlled rate over an extended period of time, USP Apparatus 3 (flow-through cell) (figure 6) should also be considered during method development because it allows changes in the medium pH during dissolution testing [51]. It should be noted, however, that USP Apparatus 3 is not currently recognized by the JP and should, therefore, not be considered for products intended for the Japanese market [51].

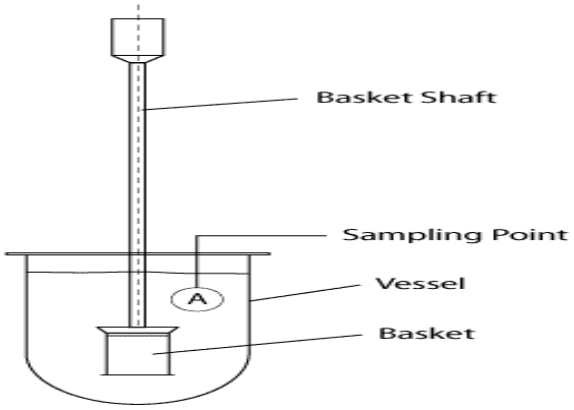

Figure 4. The rotating basket method for dissolution test (apparatus 1). Adapted from Florence and Attwood (2011) [52]

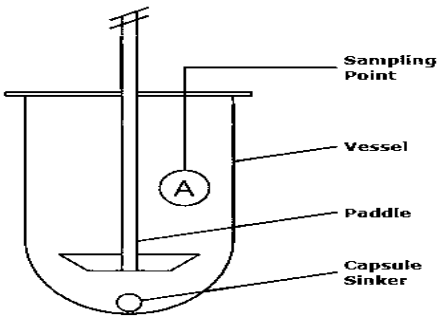

Figure 5. The rotating paddle method for dissolution test (apparatus 2). Adapted from Florence and Attwood (2011) [52]

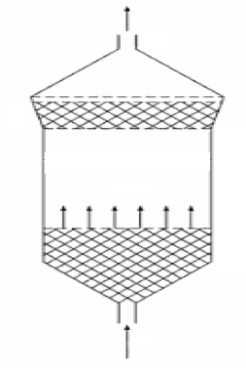

Figure 6. A recommended standard design for a flow-through cell. (The cell is cylindrical in shape and constructed of glass or other suitable material. The arrows represent the fluid from bottom to top). Adapted from Florence and Attwood (2011) [52]

Table 4 summarizes some apparatus suggested for drug release testing of various oral dosage forms:

Table 4. Suggested Apparatus for Drug Release Testing for most common Oral Dosage Forms. Adapted from Brown *et al.* (2011) [50]

Oral Dosage Form	Released Method
Oral solid dosage form (conventional)	Basket apparatus, paddle apparatus or flow-through cell
Oral suspensions	Paddle apparatus
Oral disintegrating tablets	Paddle apparatus
Chewable tablets	Basket apparatus or paddle apparatus
Powders and granules	Flow-through cell
Liquid-filled capsules	Paddle apparatus

2.2.3. Impurities, Degradation Products and Residual Solvents

Impurities, degradation products and residual solvents, deriving from the manufacturing process or originated during storage, as well as, starting materials relevant to the drug substance used for the clinical trial, should be stated [53].

During the development of a new drug, a comprehensive set of stability tests is required, so the manufacturer should gather a thorough understanding of the characteristics of the product and how they are influenced by environmental conditions. Additionally, through the results from this initial assessment of stability, the manufacturer offers an open expiry date (open shelf-life) and defines the initial storage conditions for the product. In the development phase, the following studies are performed [54, 55]:

- Forced degradation test with the following objectives:
    - To identify degradation products of the API and excipients, and to identify degradation conditions, with the aim to provide information on the development and validation of the manufacturing process and storage conditions of the product;
    - Identify environmental factors that could potentially influence the

stability of the drug, and to define which packaging materials are most suitable to protect the product.

- Photostability test: conducted in at least one batch of pharmaceutical product, according to the requirements of the "*Guideline ICH Q1B*", with the aim to demonstrate that the light does not cause changes in the product [56].
- Stability in use: this study should be undertaken whenever evaluating the stability of a multidose container, simulating normal use, and aims to determine the shelf-life after opening.

Forced degradation studies are described in various international guidelines. The International Committee for Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use has published a set of guidelines, which have been discussed, agreed and adopted by the American, European and Japanese regulatory authorities. In the majority of cases, the ICH guidelines only apply to the marketing applications for new products, i.e., they do not apply during clinical development. However, since the conditions used for forced degradation are only defined in general terms, it is possible to apply them for developing stability, indicating methods during clinical development. The same forced degradation conditions can then be applied to the drug substance during development and commercialization [57]. The ICH guidelines that are applicable to forced degradation studies are:

- ICH Q1A – Stability Testing of New Drug Substances and Products [55];
- ICH Q1B – Photostability Testing of New Drug Substances and Products [56];
- ICH Q2B – Validation of Analytical Procedures: Methodology [58].

In ICH Q1A, section 2.1.2 (Stress Testing), there are recommended conditions for performing forced degradation studies on drug substances and drug products. The recommendations are to examine the effects of temperature (above that for accelerated testing, i.e., >50°C), humidity (≥75% relative humidity), oxidation and photolysis. Testing in solution should also be performed across a wide pH range, either as a solution or suspension. These samples are then used to develop a stability-indicating method [55].

However, ICH guidelines do not give any guidance as to how much degradation is required in forced degradation studies. If too little stress is applied, some degradation pathways may not be observed, which would not challenge the method's ability to detect and monitor degradation products during stability testing. If too much stress is applied, then unrealistic degradation products may be observed and the result of analytical method may be unsuitable for detecting degradation products formed during stability testing. Thus, the conditions should be chosen carefully, so that the amount of degradation of the drug substance produced during forced degradation is neither too excessive nor too little [57].
Simon [57] suggests that HPLC with mass spectrometry detection can provide valuable information on the structural changes occurring as a result of degradation, which can help in the interpretation of the degradation pathways.
In analytical terms many drug substances have poor aqueous solubility and require addition of an organic co-solvent to maintain solubility [59]. Solution samples are easier to handle and lend themselves to increased accuracy in analytical testing. However, ICH Q1A does not state that solutions are required for forced degradation studies, but, in turn, suspensions will tend to undergo a slower rate of degradation than a solution, and typically require whole vial analysis to reduce errors associated with sub-sampling a suspension [57]. The use of co-solvents prevents all of these issues, but, however, the use of organic co-solvents can lead to the following [57]:

- Increased or decreased rate of degradation;
- Reaction products between drug substance and the organic co-solvent;
- Peaks in the chromatogram arising from the organic co-solvent.

Forced degradation of drug substances has the potential to form many more degradation products than those observed to form during stability testing. However, this minimizes the potential for not detecting the actual degradation products formed during stability testing. Thus, if appropriate forced degradation studies have been performed, the method can be considered to be stability indicating. The results from the forced degradation studies can be used to investigate the mechanism of degradation for a drug substance on storage. In such cases, the absence of observed degradation products demonstrates that the drug substance is stable to degradation under the storage conditions [57]. This understanding can be used to define the appropriate packaging, minimizing the degradation of the drug substance [57].

2.2.4. Types of Conventional Formulations

Dose is a major factor controlling the type of formulation and processing [14].
In this topic, the main types of conventional formulations will be briefly discussed, and, in the following topic, a major focus will be given to some new drug delivery systems.

2.2.4.1. Liquid Formulations

Liquid formulations account about 30% of products in the United Kingdom market (and probably in other developed countries) and, because they are easy to swallow, are favoured for paediatrics and geriatric use [14]. An aqueous solution is the simplest formulation to produce, but more complex, such as suspensions or emulsions systems, will be required if the drug is poorly soluble. Another advantage is that liquid formulations have myriad possibilities of innovative drug delivery systems. One of the most desirable features of liquid formulations, particularly the solution forms, is the relatively lower importance of bioavailability considerations, as the drug molecules are already in the dispersed phase, removing many rate-limiting steps in the absorption of drugs [60].
Liquid formulations are probably the most versatile systems, since they can be used for various routes of administration. However, liquids are difficult to transport and container breakage, if glass is used, can result in catastrophic loss, and, consequently, increase costs. Another disadvantage, is the fact that the non-sterile aqueous liquids are liable to microbial growth and, therefore, require the addition of antimicrobial preservatives [14].
A special requirement for parenteral or injectable formulations is that they must be sterile, apyrogenic and free from visible particulate contamination [61]. Generally, sterilization is conducted using a thermal sterilization method, such as autoclaving at 121ºC for 15 minutes for aqueous liquids [61]. This is a severe challenge to the drug's chemical stability, and studies must be conducted to ensure that degradation does not occur, specially for thermolabile compounds, which requires specialised sterilization methods [14].
Since suspensions contain a solid drug as a disperse phase normally in an aqueous-based liquid, they are physically unstable, because the solid deposits. So, the formulation must be design to limit this phenomenon. Suspension stability is also determined by the drug's

particle size, and limits will be required because small variations can induce physical instability if the formulation is not robust. In addition to antimicrobial preservatives to prevent microbial growth, flavours and sweeteners may be required to achieve the desired organoleptic properties [14].

2.2.4.2. Solid Formulations

Solid formulations, such as tablets and capsules, are perhaps the most common formulations marketed in the world, mainly because they have great advantages in providing the dose in a discrete unit form that is stable, easily produced, transported and, above all, easy to administered [14, 62, 63]. The tablet is favoured because it is marginally cheaper to produce and slightly more stable under in-use conditions [14]. The simplest solid dosage form is the drug powder itself, a presentation mode that is still used for some antacid preparation and pediatric use.

There are three main methods of tablet manufacture, with choice depending on the dose and the drug's physical properties such as compressibility and flow (see figure 7). A tablet with a large API dose (>100 mg) and a good flow and compressibility properties may be directly compressed into a tablet after mixing with suitable excipients [14], that are available to allow production of tablets at high speeds without prior granulation steps. These directly compressible excipients have desirable properties of flow and compressibility, and generally are substances such as lactose, sucrose, dextrose, or cellulose. Direct compression avoids several problems associated with wet and dry granulations. However, the intrinsic physical properties of the excipient are highly critical, and minor variations can alter flow and compression characteristics making them unsuitable for direct compression [63].

When physical properties are not ideal, some form of pre-treatment, such as granulation, is necessary [64]. The purpose of wet and dry granulation is to improve flow and density of the mixture and to enhance its compressibility [63]. In wet granulation, the drug is mixed with a diluent and then a solution of a polymeric binder is added during continuous mixing to form a wet powder mass. The mass may be passed through a sieve to produce reduced agglomerates. To achieve a homogeneous size, the powder is dried in hot air, and the

resulting granules are blended with other excipients such as disintegrant, diluent and lubrificant. The final mixture should flow easily in order to be compressed into a tablet [14]. Dry granulation involves the compaction of powders at high pressures. These compacts are then milled and screened to form a granulation of the desired particle size. The advantage of dry granulation is the elimination of heat and moisture in the processing [63].

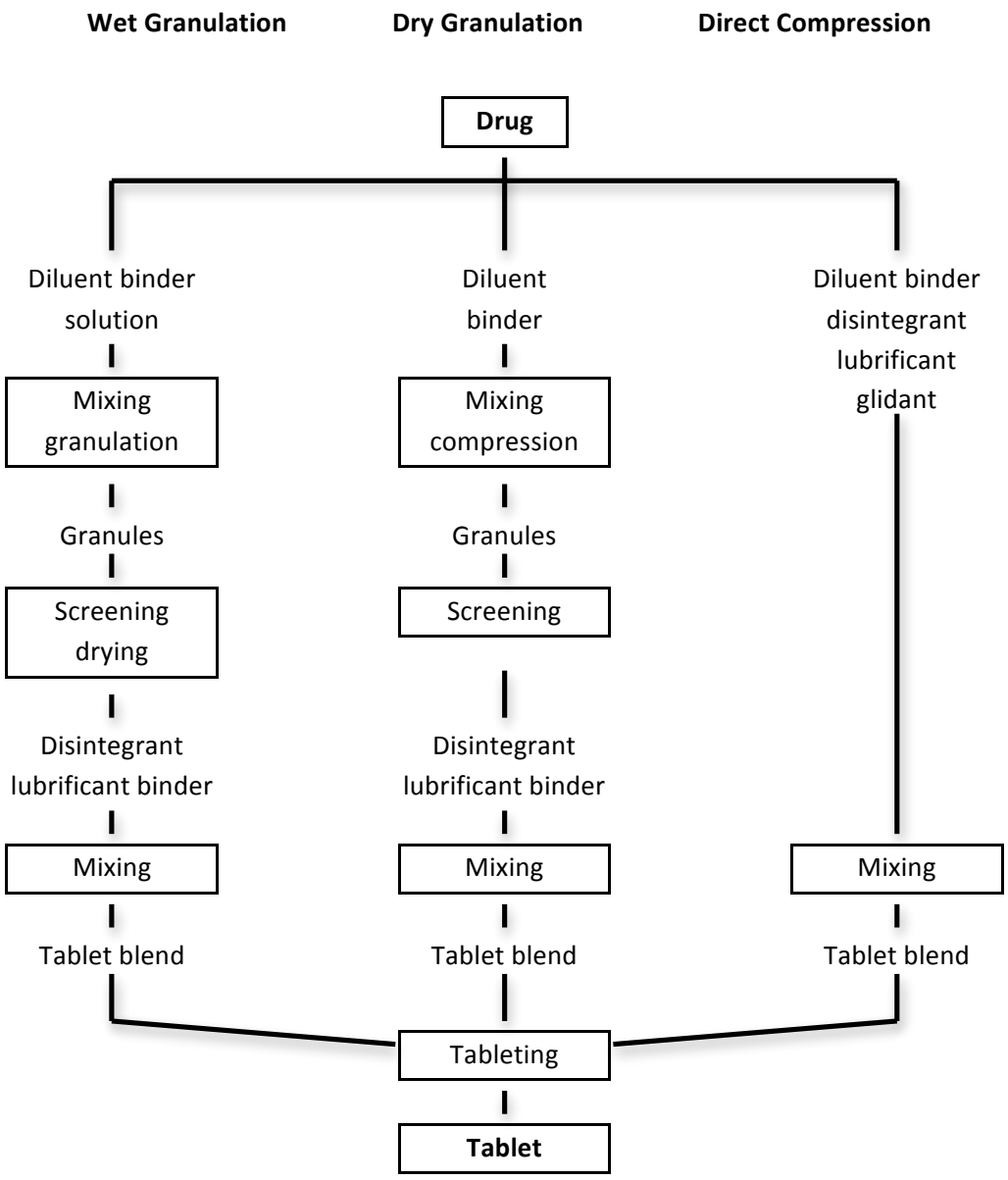

Figure 7. The tablet production fluxogram. (Process stages are shown in boxes). Adapted from Halbert (2009) [14].

Despite representing one of the oldest pharmaceutical techniques, coating of dosage forms is still frequently used in pharmaceutical manufacturing. The aims of coating range from simply masking the taste or odor of drugs to allow control release formulations [65]. The traditional tablet coating is a sugar coat applied in stages as described in tablet. First, the tablet surface is sealed to prevent the ingress of water, and then a subcoat of an aqueous polymeric or sucrose solution is added to smooth the surface of the tablet. This process can be repeated until the desired size and shape is achieved. Finally, a coloured sugar coat is applied and wax polish, and the company logo may be printed on the tablets, which can present an expensive and laborious process. Therefore, it has largely been replaced by film coating, which utilizes a coat of a polymer dissolved in a suitable solvent [14]. This process generally does not affect the tablet performance, but could increase the manufacture process, and could also difficult blinding process when comparative clinical trial trials are performed [14].

2.2.4.3. Semi-solid Formulations

Semi-solid formulations are normally employed for the topical applications of drugs and mucous membranes in order to provide a local action. Drug can be either dissolved or suspended in the formulation. The semi-solid formulations most commonly used are emulsions, ointments and pastes.

Emulsions are a two-phase system consisting of water and oil, that can be classified in two types: the oil-in-water emulsion (which has oil droplets dispersed in a continuous aqueous phase) and the water-in-oil emulsion (which as water droplets in a continuous oil phase) [66]. Oil is often chosen for its ability to solubilise the drug and its compatibility with the route of administration [14]. However, an oil-in-water emulsion is thermodynamically unstable and will tend to separate into two distinct liquid phases, being necessary the use of emulsifying agents to reduce interfacial tension between the oil and the water, in order to avoid coalescence [66]. Emulsions are also particularly sensitive to adverse storage conditions, such as change in temperature, and, therefore, may require specialised storage. Furthermore, as with all aqueous-based preparations, it will be required an antimicrobial preservative, as well as an antioxidant to prevent rancidification of their oil [14, 66].

For semi-solid products, any change in the preservative may affect the quality of the product. If any quantitative or qualitative changes are made in the formulation, additional testing should be performed. No *in vitro* release documentation or *in vivo* bioequivalence documentation is needed for preservative changes [67].

2.4.5. New Drug Delivery Systems for Drug Administration

New insights in molecular biology, material sciences and biomedical sciences have produced a portfolio of new systems, materials and approaches with the potential to treat disease. The associated research has crossed physical, technological, chemical, biological, mathematical and biomedical sciences in the search for delivery systems that behave selectively against complex targets at the true target site, delivering optimal concentrations of drug at the right, and over an optimal period of time [68].

Figure 8 schematically illustrates the long march of controlled drug delivery systems from the macroscopic size range nano-domain.

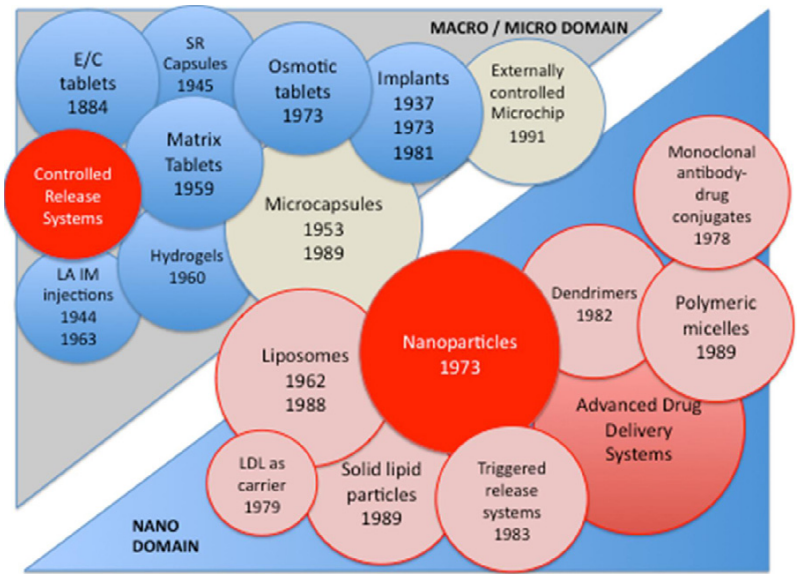

Figure 8. A schematic representation of the progress from macro and micro delivery systems to the nano-domain over the period from the 19th century to today. (The dates given represent early discovery and significant events after discovery when there are several dates). Adapted from Crommelin & Florence (2013) [68].

Various new drug delivery systems have been developed to answer the question: How to get the drug to the right place in the body and how to control the release of the drug to prevent overdoses?
In the next subtopics some of the most recent drug delivery systems will be discussed.

2.4.5.1. Osmotic Systems

Much research has focused on developing drug candidates for oral administration capable of yielding an *in vivo* plasma concentration effective and reproducible, since oral administration is considered the most convenient. However, as most drugs have a poorly oral absorption or require a high frequency of administration, the modified release formulations are an effective method for optimizing the bioavailability and blood concentration profile over time (see the advantages and disadvantages use of modified release forms in table 5) [62]. According to the Portuguese Pharmacopoeia VIII there are different release profiles of drugs, such as pharmaceutical dosage form of conventional release, pharmaceutical dosage form of modified release, pharmaceutical dosage form of sustained release, pharmaceutical dosage form of delayed release and pharmaceutical dosage form of sequential release [69].
The osmotic systems are currently one of the main types of modified release systems, which use the principle of osmotic pressure to promote the drug release, that consists in the presence of an osmotically active core containing the drug, covered by a rigid membrane, semi-permeable (permeable to the water but not to the drug), usually with a well-defined size hole, allowing release of the drug. In an aqueous medium, the water penetrates through the semipermeable membrane into the core of the system, at a rate dependent on the composition and thickness of the membrane, increasing the internal pressure. The fact that the membrane is semipermeable, meaning that the water enters the membrane at a constant speed, and the drug outs through the orifice also at a constant release rate during a prolonged period of time, ensuring a constant release of the drug [62, 70, 71].

Table 5. Advantages and Disadvantages of Modified Release Formulations

Modified Release Formulations	
Advantages [62, 72]	**Disadvantages** [62]
Simplification of the dosage scheme	Possibility of rupture of the system and consequent excessive release of drug
Decrease the possibility of errors or forgetfulness	Expensive
Minor fluctuations in plasma concentrations	
Reduced gastrointestinal irritation	
Decrease of adverse reactions or toxic effects	
Absorption more effective	
Release of the drug at a determined time	

2.4.5.2. Matrix Systems

Nowadays, the matrix systems are perhaps the most used system [73], due to intrinsic advantages such as versatility, efficiency, low cost, production equipment and techniques [74]. In those systems, the drug is incorporated in a structure formed by chains of one or more chemical substances that act as release modulating agents, either in the form of solution or dispersion, in order to regulate the speed and location of drug release [73, 74], providing a duration of therapeutic activity sustained over time and/or specific delivery of drug at a particular tissue or cell population [74]. The inert matrices are comprised of insoluble materials that cause solid porous structures in which the drug is dispersed by maintaining the apparent surface over the release process [74].

Drug release is quite complex, since it must be considered erosion of the matrix during the process. The main factor that regulate drug release are the solubility and diffusivity through the polymer [73].

Most matrix systems resort to careful selection of several types of excipients in order to modulate the transfer of drugs. Thus, the polymers are versatile agents to perform these functions [75].

The matrix systems can be classified as mineral matrix, hydrophilic matrix, inert matrix, lipidic matrix and biodegradable non-lipid matrices [74], and may appear as tablets, but most commonly in microcapsules. The micro and nanoparticles are often prepared with inert matrices [73].

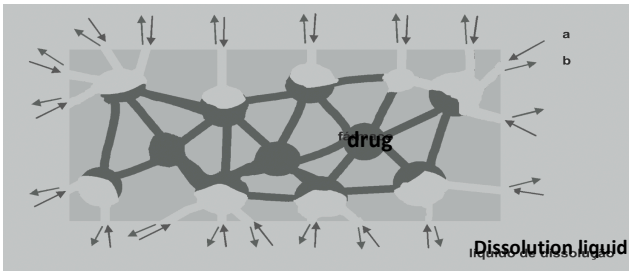

Figure 9. Release of Drugs in Matrix Systems: a) dissolving liquid penetration into the pores of the matrix system, b) canaliculi by slow diffusion of dissolved drug to the exterior. Adapted from Lopes *et al* (2011) [74]

2.4.5.3. Mucoadhesive Systems

The oral bioavailability of many drugs can be limited by the residence time of pharmaceutical dosage forms through the gastrointestinal tract [76, 77]. Once administered, dosage forms must undergo disintegration, dissolution of the drug employed and subsequent absorption through the mucosa. Since each drug has a region where absorption is preferred, the residence time of the dosage form/drug in this region is critical [78].

The mucoadhesion has been proposed as a strategy to increase the residence time of pharmaceutical dosage forms at a specific area and to reduce its variability [78], as well as to maximize systemic absorption of drugs and the performance of topically acting agents [79]. Thus, the therapeutic effect of the drug can be increased.

The term mucoadhesion refers to the interaction between natural or synthetic macromolecules and mucus layer that lining the mucosa of the gastrointestinal tract, and the term bioadhesion is used when the interaction occurs at the epithelial surface. The

profile of a mucoadhesive formulation intended for oral administration depends on the strength of interaction of the components of this formulation with the mucus [76].

Most research has focused on mucoadhesion in the stomach and small intestine, with promising results observed from *in vitro* studies. However, the data obtained in human studies revealed lack of success of mucoadhesion, mainly because of the complex nature of the human gastrointestinal tract. In addition, most *in vitro* models provide little resemblance to the situation *in vivo* in several aspects such as motility, pH, thick mucus and sputum volume, presence of food and enzymes. Once the motility of the colon is lower, mucoadhesion can be most successful in this part of the gastrointestinal tract. [78]. Nevertheless, further studies in animals and humans, as well as pharmacokinetics studies, are needed to determine absorption of the drugs based in mucoadhesive systems.

Some authors have classified such systems as gastroretentive systems.

2.4.5.4. Gastroretentive Systems

The bioavailability of drugs with an absorption window in the upper small intestine is generally limited with conventional pharmaceutical dosage forms. The residence time of such systems and, thus, of their drug release into the stomach and upper intestine is often short. To overcome this restriction and to increase the bioavailability of these drugs, controlled drug delivery systems with a prolonged residence time in the stomach can be used. Approaches to achieving prolonged residence times of the devices in the upper part of the gastrointestinal tract include the use of bioadhesive, size-increasing, floating and magnetic drug delivery systems [80, 81].

The most common classification of gastroretentive systems is according to the principle of gastroretention applied (see table 6) [82].

Such drug delivery systems (high-density, floating, unfolding, mucoadhesive, magnetic systems, etc.) are interesting and present their own advantages and drawbacks. But an important feature to take into account is the stomach physiology, including motility variables (gastric emptying, small intestinal and colonic transit rates, postprandial frequency of contractions), and also pathological conditions such as diabetes that can

profoundly affect the physiological gastric state, so that specific refinements to formulations may prove necessary [81].

Table 6. Classification of Gastroretentive Systems and their Characteristics

System type	Characteristics
High-density systems	Based both on the anatomy of the stomach and in the gastric contents, that have a density similar to the water [82]. If administering pharmaceutical systems with a density greater than 2.5 g/cm^3, it is conceivable that these systems are in the fundus of the stomach, where it is easier to be expelled by the contraction waves [83].
Floating systems	These have a bulk density lower than the gastric content. They remain buoyant in the stomach for a prolonged period of time, with the potential for continuous release of drug [81]. These systems generally consist of hydrophilic polymers that swell upon contact with aqueous solutions. The combination of processes of swelling and erosion of the gelled layer that is formed, allow control of drug release and also the fluctuation of the pharmaceutical system [82].
Expandable systems	These unfolding systems are made of biodegradable polymers. The concept is to make a carrier, such as a capsule, incorporating a compressed system, which extends in the stomach. In spite of their interesting characteristics, expandable systems have drawbacks. Storage of such easily hydrolysable, biodegradable polymers is problematical [81].
Mucoadhesive or bioadhesive systems	In mucoadhesive systems occurs interaction between natural or synthetic macromolecules and mucus layer that lining the mucosa of the gastrointestinal tract, increasing the residence time.
Magnetic systems	This system is based on a simple idea: the dosage form contains a small internal magnet, and a magnet placed on the abdomen over the position of the stomach [81].

2.4.5.5. Transdermal Systems

The delivery of drugs through the skin provides a convenient route of administration that is often preferable to injection because it is noninvasive and can typically be self-administered, resulting in a significant reduction of medical complications and an improvement in patient compliance. Unfortunately, a significant obstacle to transdermal drug delivery is the resilient barrier that the epidermal layers of the skin, primarily the stratum corneum, present for the diffusion of exogenous chemical agents [84].

Transdermal delivery use the skin as the application site, introduces the drug for transport into the circulatory system, keeping a constant plasma concentration [85, 86]. The delivery of drugs through the skin provides a convenient route of administration that bypasses the gastro-intestinal tract, first-pass metabolism, and many of the complications associated with injectable drugs [85].

Furthermore, these drug delivery systems have another advantages, such as [86]:

- Prevents degradation of the drug in gastrointestinal tract due to enzymatic conditions;
- Promotes the absorption of drugs at therapeutic doses and maintain adequate plasma concentrations constants;
- Reduces the frequency of administration;
- Avoids the first-pass metabolism;
- Allows immediate discontinuation of the administration;
- Enables a constant drug plasma levels;
- Promotes adherence to therapy;
- It is a noninvasive drug delivery system.

However, because the skin is extremely effective at protecting the body from external pathogens and toxins, transdermal delivery systems must be designed to circumvent its barrier properties [85].

The drugs candidates for transdermal route of administration must have physicochemical characteristics suitable for penetrating the skin barrier, i.e. high pharmacological potency, non-irritating, low polarity, non-ionized form, low-mass molecules and appropriate partition coefficient [86].

2.4.5.6. Hydrogels

The properties of hydrogels, in particular their high biocompatibility and water sorption uptake, make hydrogels very attractive in drug delivery and biomedical devices [87]. However, these favorable features of hydrogels are compromised by certain structural limitations, such as those associated with their low mechanical strength in the swollen state [88].

Hydrogels are a hydrophobic polymeric network of three-dimensional structures consisting of single chain or multiple chains of polymers (monomers) being cross-linked [87], making them insoluble in water, due to ionic interaction and hydrogen bonding. The network structure of hydrogels can be macroporous (hydrogels that release the drug entrapped inside the pores through a mechanism dependent on drug diffusion coefficient), microporous (the drug release are made by molecular diffusion and convection) or nonporous (the release of drug is only by diffusion mechanism) [89].

These hydrogels, being biocompatible and biodegradable in nature, have been used in the development of nano-biotechnology products and have important applications in the field of controlled drug delivery, gaining attention as intelligent drug carriers [89].

2.4.5.7. Micro and Nanoparticles

The reason behind the failure of conventional delivery systems in reaching the brain is the blood brain barrier composed of tight tissues making it impervious to outside agents [90]. Nanotechnology has the potential to address and resolve this challenge and make brain a reachable target for drug delivery systems, by combining unique elements of size, surface activity and charge of nanostructures [91]. Micro and nanoparticles have brought new ways in diagnostics and therapeutics [90]. Nanodelivery of drugs is predicted to reduce collateral damage, extend the drug's availability and effectiveness at the site, and reduce toxicity and cost with a high pay-off load [91].

Micro and nanoparticles are defined as solid systems based on polymers (synthetic, semi-synthetic or natural), or other materials, of biodegradable nature or not, with dimensions on the order of micrometers and nanometers, respectively, and serving as a vehicle for drugs.

As regards the structure, the term micro and nanoparticle comprises two more specific types: the micro and nanospheres, and micro and nanocapsules [92].

As noted by several authors, the nanoparticulate systems are promising as active vectors due to their ability to release drugs [93], and their subcellular size, allowing relatively higher intracellular uptake in comparison with other systems. In addition, they can improve the stability of drugs, and may be biocompatible with the tissue and cells [94].

The nanoencapsulated systems include high drug encapsulation efficiency due to the optimized drug solubility in the core, low polymer content compared with other nanoparticulate systems, such as nanospheres, and protection against degradation factors such as pH and light, and the reduction of tissue irritation due to the polymeric shell [94, 95]. Nanocapsules can be compared to vesicular systems, where drug is confined to a cavity consisting of a liquid core surrounded by a polymeric membrane [92, 94].

The cavity can contain the drug in liquid or solid form, or as a molecular dispersion [95, 96]. The reservoir may be lipophilic or hydrophobic according to the preparation method and raw materials used. Furthermore, taking into account operational constraints preparation methods, nanocapsules can also carry the active substance on its surface or impregnated in the polymeric membrane [92, 94].

2.4.5.8. Vesicular Systems

During the past decade, formulation of vesicles, as a tool to improve drug delivery, has created a lot of interest amongst the scientist working in the area of drug delivery systems. Vesicular systems, such as liposomes and niosomes, provide an alternative to improve the drug delivery [97]. These systems, especially liposomes, have generated a great interest, since they can be used as sensitive containers that respond to external stimuli to delivery the drug, such as pressure, pH, temperature, or concentration changes in the medium, producing modifications in their structure. The control of the nanostructure-particle size and size distribution, membrane morphology, and supramolecular organization of these self-assembled systems is of great importance for their application in drug delivery [98].

2.4.5.8.1. Liposomes

Liposomes are a well-known and well-used vesicular delivery system, formed by a lipid bilayer surrounding an aqueous solution. They can deliver both hydrophilic and lipophilic actives to their target across the stratum corneum [99, 100]. The diameter and number of layers depend primarily on the manufacturing process, with the multilamellar, large unilamellar and small unilamellar vesicles (table 7) being the most commonly used [101].

Table 7. Classification of Liposomes according to their Diameter. Adapted from Matos & Moutinho (2011) [101]

Type of liposomes	Diameter (nm)
Multilamellar Vesicles (MLV) (Also known as conventional liposomes or first generation liposomes)	500-5000
Large Unilamellar Vesicles (LUV)	100-500
Small Unilamellar Vesicles (SUV)	20-100

Many reviews and book chapters have focused on the application of liposomes for drug delivery, gene therapy, and immunization.

It is important to highlight the many advantages of liposomes, such as [101-104]:

- To protect the drug from potential instabilities;
- To promote a constant concentration;
- To promote gradual and controlled release of the drug;
- To decrease toxicity;
- To target specific cells or organs;
- To incorporate both hydrophilic substances or lipophilic substances;
- To decrease the number of doses;
- To reduce the total time of the treatment, which leads to an increase in therapy acceptance by the patient.

Classically, liposomes can be prepared from mixtures of amphiphilic lipid (phospholipid and cholesterol) extracted and purified natural or from synthetic lipids [101, 102, 105].

The cholesterol, as well as triglycerides or propylene glycol esters, make the membrane of liposomes more waterproof, allowing sustained release of the drug. The control over the rate of drug release by the vesicle can also be achieved by manipulating the size, surface area and membrane fluidity [101].

Liposomes are predominantly removed from circulation by phagocyte cells of the reticuloendothelial system, which may lead to accumulation of a large extent in organs like liver and spleen. This biodistribution pattern can be used for passive targeting of diagnostics to these organs, but information on biodistribution is, therefore, important for drug targeting by liposomes [102, 105].

Liposomes given intravenously usually interact mainly with opsonins, which bind to the surface of vesicles and mediate their endocytosis by the mononuclear phagocyte system (macrophages). The rate of liposome clearance from blood circulation will, therefore, depend on the ability of opsonins to bind to the liposome surface [102].

On the other hand, there are also constraints in the commercialization of liposomal preparation. First, the lipids needed for their preparation are scarce, should be of high purity and, hence, expensive. Secondly, liposomal preparations are inherently unstable [103] and require special storage conditions even when the products are freeze-dried. As a result of this stability problem, the dosage forms are limited to injection (freeze-dried) powders for reconstitution immediately before use [102].

2.4.5.8.2. Niosomes

Niosomes are multilamellar vesicular structure of non-ionic surfactants (instead of phospholipids), similar to liposomes [97, 106], widely studied as an alternative tool to liposomes [97].

Niosomes contain mainly two types of components *i.e.*, non-ionic surfactant and the additives. The non-ionic surfactants form the vesicular layer. Additives used in niosome preparation are usually cholesterol [106] that improves the rigidity of the bilayer, affecting it fluidity and permeability. This carrier system protects the drug molecules from the

premature degradation and inactivation due to unwanted immunological and pharmacological effects.

As a drug delivery system, niosomes are more stable and cheap compared to other vesicular systems. In recent years, niosomes have been extensively studied to delivery of various therapeutically active moieties, such as gene delivery, anti-cancer agents, hormones, antigens, anti-inflammatory agents, and anti-infective agents [97, 106, 107]. Besides this, niosome has been used to solve the problem of insolubility, instability and rapid degradation of drugs [108, 109]. While selecting a suitable drug for niosomal drug delivery, it should be kept in mind that niosomes encapsulating hydrophobic drugs and macromolecules are more stable than niosomes encapsulating low molecular weight drugs. These factors also affect niosome stability *in vivo* [106].

The niosomes, as a drug delivery system, offer the following advantages [97, 106-109]:

- Can be utilized in the delivery of wide variety of drugs as it has capability to entrap hydrophilic, lipophilic, as well as amphiphilic drugs;
- Controlled and sustained release of drugs due to depot formation;
- Greater bioavailability than conventional dosage forms;
- Effectively used in targeting drugs to various organs;
- More stable than liposomes;
- Increase the permeation of drugs through the skin;
- Administrated *via* various routes like oral, parenteral and topical *etc;*
- Biodegradable, biocompatible and non-immunogenic to the body;
- Easy handling, storage and transportation;
- Protect the drugs from biological enzymes and acid, leading to the drug increase;
- Penetration enhancers in the ocular drug delivery system cause no tissue irritation and damage.

2.4.5.9. Microemulsions and Nanoemulsions

The application of microwave technology to the preparation of drug products has become increasingly important. The micro and nanoemulsions are very promising therapeutic systems, presenting technological advantages that enable the optimization of pharmaceutical operations, such as emulsification and solubilization of drug, avoiding problems relating to thermodynamic instability of classic emulsions [110, 111].

Microemulsions are clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. These systems act as drug delivery vehicles by incorporating a wide range of drug molecules [112].

Until now, microemulsions have been shown to be able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Furthermore, it is possible to formulate preparations suitable for most routes of administration. But fundamental work to characterize physicochemical behavior of microemulsions needs to be performed. [112, 113].

Nanoemulsions are a class of colloidal systems liquid-liquid, transparent or translucent, with very small uniform droplets, between 5 and 200 nm [114-116].

According to the preparation of such systems, the drug is dissolved in the lipophilic part of the nanoemulsion, i.e. oil and the water phases can be combined with a surfactant or co-surfactant, and then added at slow rate with gradual stirring until the system is transparent. Finallys, ultrasonicator is used to achieve the desired size range for dispersed globules, allowing equilibrium [115].

Nanoemulsions have several advantages, and many of them are common to the microemulsions [110, 115, 116]:

- Increase the rate of absorption;
- Helps solubilize lipophilic drug;
- Provides aqueous dosage form for water insoluble drugs;
- Increases bioavailability;
- Various routes, such as tropical, oral and intravenous, can be used to deliver the product;
- Provides protection from hydrolysis and oxidation of drugs;
- Same nanoemulsions can carry both lipophilic and hydrophilic drugs;

- Improves the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

However, there are also some disadvantages regarding nanoemulsions [115]:

- Large concentration of surfactant and co-surfactant is necessary for stabilizing the nanodroplets;
- Nanoemulsion stability is influenced by environmental parameters, such as temperature and pH.

2.4.5.10. Solid Lipid Nanoparticles

Polymeric nanoparticles, which are generally made with suitable biodegradable polymers, have been shown to prolong the release of the incorporated drugs [117] and have been developed as an alternative to liposomes [118]. Solid Lipid Nanoparticles (SLN) consist on a solid matrix at body temperature, with physiological lipids, coated with surfactants, and with sizes between 50 and 1000 nm [119]. SLN attracted increasing attention as an efficient and non-toxic drug carrier [120, 121]. Furthermore, they can be produced to incorporate lipophilic or hydrophilic drugs [122, 123]. Their colloidal dimensions and the controlled release behavior enable drug protection and administration by parenteral and non-parenteral routes, emphasising the versatility of this nanoparticulate carrier [120, 121, 124].

2.4.5.11. Nanosponges

Nanosponges are a new class of materials made of microscopic particles with few nanometer wide cavities, in which a large variety of substances can be encapsulated within its core. Both lipophilic and hydrophilic substances can be incorporated, and one of the main advantages is solubility improving of poorly water-soluble molecules [125-127]. Also, nanosponges can be used to increase the dissolution rate and stability of drugs [125, 126].

The nanosponges are solid in nature and may be used in formulations for use by various routes of administration. For the oral administration, they can be dispersed in excipients, diluents and lubricants. For the parenteral administration they can simply be carried in sterile water, saline or other aqueous solutions [125]. For topical administration they can be effectively incorporated into topical hydrogel [128].

2.4.5.12. Modified Release by Activation of Pro-drugs

A strategy to improve several parameters of pharmacologically potent compounds is the development of pro-drugs. A pro-drug is a chemically modified version of the drug that must undergo biotransformation *in vivo* to release the active drug [129-131]. Pro-drugs provide possibilities to overcome various barriers, such as [132, 133]:

- Poor aqueous solubility;
- Chemical instability;
- Insufficient oral absorption;
- Rapid pre-systemic metabolism;
- Inadequate brain penetration;
- Toxicity and local irritation;
- Improve drug targeting.

The drug–promoiety complex (drug and promoiety are covalently linked by a chemically or enzymatically labile bound) is typically pharmacologically inactive developed to cross a barrier, which can be any obstacle that prevents optimal (bio)pharmaceutical or pharmacokinetic performance [133, 134]. The ideal pro-drug yields the parent drug with high recovery ratios, with release of a non-toxic promoiety (figure 10) [132, 133].

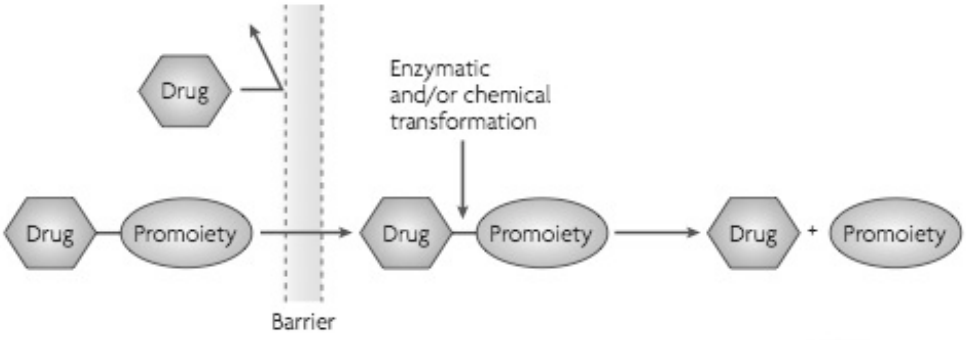

Figure 10. Representative Illustration of the Pro-drug Concept. Adapted from Rautio *et al* (2008) [133]

According to Rautio *et al*, currently 5–7% of the drugs approved can be classified as pro-drugs, and approximately 15% of all new drugs approved in 2001 and 2002 were pro-drugs [133].

2.4.5.13. Modified Release by Inclusion of the Drug in CDs

The molecular encapsulation of drugs by CDs is very advantageous from a technological and biological standpoint, because allows to modify the physical, chemical and biopharmaceutical properties of the drug, being easily to produce and less expensively than the encapsulation of drugs by other methods [135-137].

CDs are considered the most important supramolecular structures, because of their capability to form inclusion complexes with a variety of molecules, including inorganic, organic, or organometallic molecules [138].

The CDs have the ability to release the drug at its target site, like brain, colon or specific cells, when the drug is covalently bonded to the CD, preventing the dissociation of the drug-CD complex before reaching the specific site of action [137, 138].

2.4.5.14. Modified Release by External Activation

The concept of "targeting of a drug", suggested by Paul Ehrlich (physiologist and Nobel Prize in Medicine, 1908) in 20th century, consider a hypothetical "magic bullet", as an entity integrating two components: the first should be able to recognize the target and bind to it, while the second should provide a therapeutic action in that target [139]. The need for delivery of drugs with efficacy and safety has been the driver force for development of new systems for controlled drug release [140].

The preparations for controlled release of drugs may [141]:

- Maintain the concentration of drug in the desired therapeutic range by a single dose;
- Target release, reducing its systemic distribution;
- Preserving drugs that are rapidly eliminated by the body;
- Increase efficacy and safety, improving patient comfort and adherence to therapy.

There are three major mechanisms by which drugs may be released from a system: diffusion, degradation, and expansion followed by diffusion [141, 142]. Dissolution or degradation-controlled drug release is based on dissolution or degradation of the polymer's membrane that encapsulate the drug, or polymer's matrix that contain the drug [142].

It is also possible to design a system for drug release, which it is unable to release its contents before being placed in a suitable biological environment - controlled release systems for expansion. These systems are initially dry, and when introduced in the body they expand by absorbing water or body fluids [141]. Most materials used in controlled release systems by the expansion are based on hydrogels. Such hydrogels can show a response to the external or internal stimuli, such as temperature and pH. Electrically responsive hydrogels (normally consist of pH sensitive polyelectrolytes) have also been recently developed [143, 144]. It is important to note that such systems, environmentally sensitive or "smart" drug release, is only achieved when the polymer expands. [141, 143].

2.4.6 Biopharmaceuticals

A biological product is defined as "a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or any other trivalent organic arsenic compound, applicable to the prevention, treatment or cure of a disease or condition of human beings" [145]. The characterization of a biotechnology product is more complicated and may include the determination of secondary and tertiary structures, degree of glycosylation, biological activity, isoform activities, impact of truncation of the molecule, amino acid sequence changes, pegylation, presence of neutralizing antibodies, and immunogenicity of the compound [40].

The manufacture of biological medicinal products involves certain specific considerations arising from the nature of the products and the processes, and some particular precautions are needed. Unlike conventional medicinal products (high degree of consistency), the production of biological medicinal products displays an inherent variability since it involves biological processes and materials, such as cultivation of cells or extraction of material from living organisms [145, 146].

The manufacturing processes for biologics, typically derive from living systems, which can be complex [147]. Biological products can be isolated from naturally derived sources or manufactured using bacteria, yeast, fungi, insect cells, plant cells, mammalian cell cultures, transgenics, and other system. Bioprocessing of proteins involves the integration and scale-up of upstream and downstream processing, process monitoring, optimization and control. The manufacture of proteins requires strict control of the starting raw materials, genetic engineering, expression systems, optimization of growth conditions, batch culture design, purification, protein analyses, formulation, analytical testing, stability, aseptic filling, packaging and the validation of the processes [148]. Careful monitoring of critical process variables provides product quality assurance and the understanding of how these process variables affects the final product [146, 148].

As with small molecules, the biological manufacturing process is intimately associated with the quality of the biological product. In general, biological manufacture can be divided into two main processes: upstream and downstream processing (figure 11) [147, 149, 150]. Upstream activities produce the protein of interest, usually by cell culture or fermentation [147-149]. Upstream considerations include integrity and quality of the process, cell banks, expression systems, cultivation, process/product purity, impurities, and

contaminants [148, 151]. Downstream processing refers to the separation and purification of the bulk bio-product into a form suitable for its use [150, 151]. This usually includes the purification, sterilization, and final formulation [148]. Typically, downstream processing techniques include filtration, centrifugation, precipitation, numerous chromatographic separations, and sterilization by aseptic processing, terminal filtration, or lyophilization [150, 152, 153].

The complexity associated with the manufacturing of biotechnology-derived products by the many available biotechnology processes may result in different impurity profile, which, if not controlled, could lead to therapeutic nonequivalence or immunogenic responses between similar biologics. Proving pharmaceutical equivalence between multisource biologics may be possible; however, the analytical procedures may be limited in their ability to detect heterogeneity, glycosylation, and conformational changes associated with complex biologics [148].

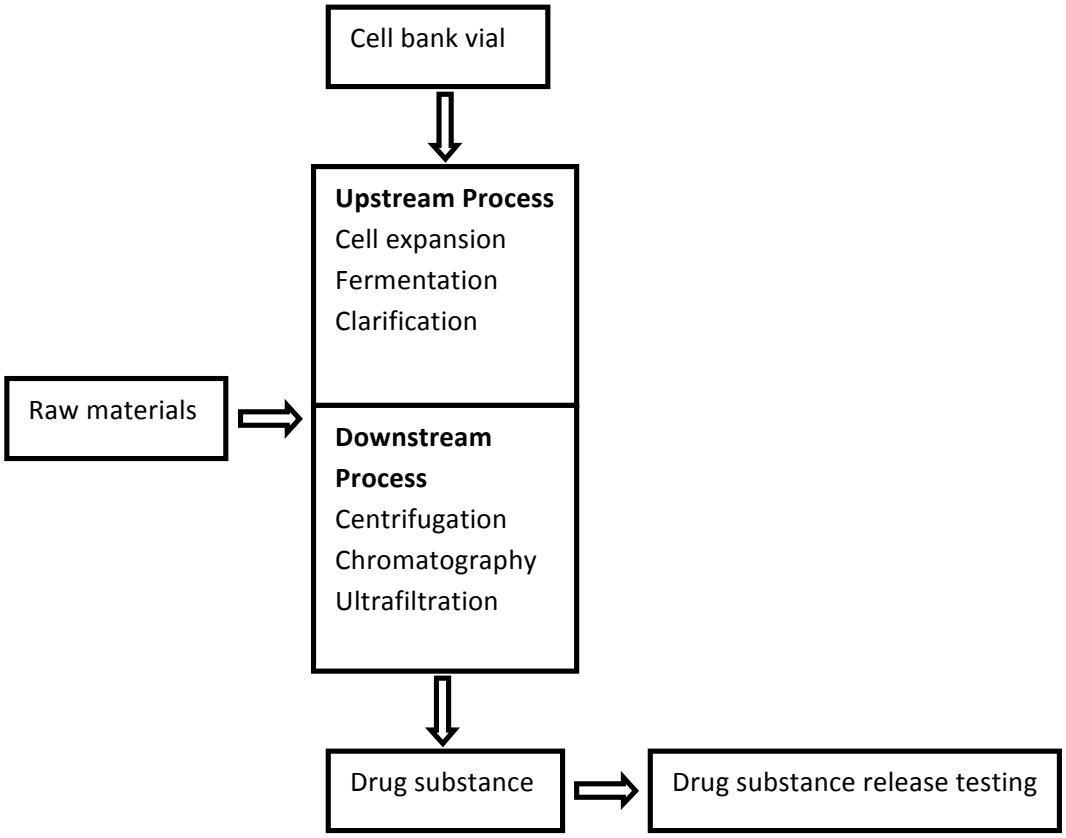

Figure 11. Biopharmaceutical Manufacturing Process. Adapted from Sekhon (2010) [147]

CHAPTER 3

Production of Investigational Medicinal Products

Investigational medicinal products should be produced in accordance with the principles and the detailed guidelines of GMP for Medicinal Products (The Rules Governing Medicinal Products in The European Community, Volume IV), although other guidelines published by the European Commission should be taken into account according the stage of development of the product [11].

The production of investigational medicinal products involves added complexity in comparison to marketed products, due of the lack of fixed routines, variety of clinical trial designs, consequent packaging designs, and the need, often, for randomization and blinding, as well as increased risk of product cross-contamination and mix up [11, 154].

The major pharmaceutical challenge is to develop the appropriate manufacturing and packaging procedures that ensure the stability and quality of the trial supplies. In addition, blinding may be required by the clinical trial protocol. The simplest trial would be active product against matching placebo in a single dose level. However, multiple dose levels or comparisons with competitors' products, increase the complexity of supplies and pharmaceutical demand [14].

3.1. Scale-up and Manufacture

The development and optimization of a formulation is an experimental phase (conducted in small batches of the material), but the overall aim of pharmaceutical development is to transform the formulation into a product that can be manufactured on a large scale. The complexity of scale-up is related to the proposed production batch size of the final product [14].

For every manufacturing operation or supply there should be adequate written instructions and written records. Records are particularly important for the preparation of the final

version of the documents to be used in routine manufacture once the marketing authorization is granted [11].

The first opportunity for a formulation scientist to scale-up the formulation is when the clinical supply requirements justify the need for larger batches [155].

Production processes for IMP are not expected to be validated duo to the extent necessary for routine production, but premises and equipment are expected to be qualified. For sterile products, the validation of sterilising processes should be of the same standard as for products authorised for marketing. Similarly, when products of biological origin are used, safety of products should be demonstrated by following the scientific principles and techniques defined in the guidance for this area. Validation of aseptic processes presents special problems when the batch size is small. If practicable, a larger number of units should be filled to provide greater confidence in the results obtained. Enhanced training should be given to operator, because filling and sealing is a manual or semi-automated operation that presents great challenges to sterility [11].

Regulatory authorities will inspect the production premises and processes to ensure that everything complies with the license application and the GMP, that must be maintained throughout the production cycle, including, suppliers and also distribution chain. In fact, GMP should only end when the product is handed to the patient [14].

The clinical formulation should have at least a year shelf-life at the specified storage condition. Attention should be taken to the time spent for clinical supplies manufacture, package and label the clinical material, and ship the supplies to the clinical site. The number of dosage strengths and placebos also will affect the manufacturing lead times [155].

Formulation development personnel, pilot plant staff, quality assurance, quality control, validation, and other support functions need to closely coordinate their activities and responsibilities [155].

3.1.1. Facilities for Production of Clinical Supplies

It is ideal to have a pilot plant that should be design to support product development activities and clinical supplies manufacture according to the GMP [156].

Clinical supplies demands increase quickly from a few hundred units to several million, especially for phase III and post-approval programs where larger batches sizes are common [157]. Companies that do not have a pilot plant capable of handling these larger batches sizes often use a contract manufacture.

A new drug may require equipment and processes at more than one plant for its production [158]. Generally, an API require its own manufacturing facility, either due to safety or the complexity of the process [158]. API's are typically manufactured in a chemical (or biological) site, whereas the filling and finishing normally are separated from the synthetic production, due to the higher level of cleanliness and different types of operations, equipment and levels of technical knowledge required by the operators [158]. Briefly, processing of clinical trials supplies for most therapeutic areas requires facilities designed to prevent cross-contamination in a controlled environment [159] .

Facilities in which cytotoxic clinical supplies are produced must be designed and engineered to protect workers and the environment. Special heating, ventilation and air conditioning systems, as well as computer-balanced room pressure differentials and strategic placed airlocks, are essential to protect the workers and the product [159, 160]. High efficiency particulate air, so called HEPA, filtration on the facility's exhaust air, neutralization of aqueous effluent, and proper disposal of organic solvent or sold wastes help protect the external environment [159].

3.1.2. Production Team

The facility needs to have proper support staff and functions to provide training, validation, maintenance, calibration, engineering, microbiological/environmental monitoring, warehousing, dispensing, housekeeping, quality assurance, and quality control [157].

All personnel whose activities are performed in areas of potential exposure to toxic substances must be trained in safe handling techniques and should receive appropriate

information, such as that provided in material safety data sheet before starting their work [159].

Facility staff should also hold periodic safety meetings to review existing procedures and identify any areas of deficiency or need for update resulting from change of process or procedure. All staff should have appropriate personal protective equipment, depending on the type of product that will handle. They should also remove accessories such as watches, bracelets, rings, etc., where toxic material may be present.

Employees who are potentially exposed to anticancer materials should participate in an appropriate medical surveillance program [161, 162].

3.1.3. Critical Aspects of Production Process

3.1.3.1. Comparators

According to regulatory definitions (EU Annex 13), a comparator medication are "an investigational or marketed product (i.e. active control), or placebo, used as a reference in a clinical trial" [11, 163]. In other words, comparators are positive control active drug supplies, and their therapeutic activity during a clinical trial essentially validates the study and establishes the base line for efficacy with which to compare the drug under study [157].

While comparison against a placebo is generally required by regulatory authorities (despite some authorities considering placebo controlled trials as non-ethical), trials can also include an active comparator when a pharmaceutical company seeks to claim superiority or non-inferiority versus the competitor drug in its marketing materials [164].

Pharmaceutical companies are pressured to conduct comparative effectiveness trials, and they should create robust strategies to select the comparator, as well as its source, in order to provide the drug in an uninterrupted manner to at all trial sites [164].

Since most clinical trials should follow a blind design, the identity of the drug and/or comparator should be blinded to the patient and investigator [157]. Thus, comparators that are easier to blind or do not require extensive development time are usually chosen [157].

3.1.3.2. Blinding of Comparators: blinding operations

Clinical dosage forms must be "blinded" with the aim to avoid bias in the clinical program. Especially for clinical phases II and III studies it is necessary to take into consideration several aspects for the appearance of the clinical trial medication, which might be contrary in regard to the appearance for marketed products [163].

Blinding is a manipulation or manufacturing technique that renders different drugs and placebo indistinguishable from each other. Thus, the NCE, comparator drug, and placebo product should have exactly the same look, feel, color, weight, shape, size, texture and taste [14, 157]. Also, the market image of the comparator must be "blinded" [157]. According to Jeatran and Clark [165], it is important to consider the drug treatments in terms of five senses, and every effort should be extended to mask any potential deviations, including those in table 8.

Table 8. Points to Consider when Blinding Treatments. Adapted from Jeatran and Clark (1998) [165]

Sight	Size, shape, color, markings, packaging, labelling
Smell	Odor of the dosage form or vehicle
Sound	A tablet inside a capsule must not rattle
Taste	Mask any unique taste of study drug and comparator
Touch	Coatings, isotonicity, viscosity, route of administration

In the ideal scenario, the positive control drug (PCD) used in a comparative, double blinded clinical study would be identical in appearance and dosing to the investigational drug. But in real life, this seldom, if ever, occurs. Marketed drugs are available in an almost infinite variety of size, shape, and colour. In addition, there are differences in the route, volume, and interval of administration, and unique packaging for many marketed drugs. Moreover, it is critical to consider stability and bioavailability to ensure that the drug is fully potent and available to have valid comparison. It is also important to ensure that the PCD and IMP are prepared to maximize patient compliance with dosage regimen. All these facts, make every new effort to prepare a blinded PCD a challenge [165].

If the NCE is a different dosage form than the comparator, a double-dummy approach is used, in which patient has to administer two products at one time, only one of them contains the active drug. The advantage is that both products are used without manipulation, but it can be very confusing for the trial participants, because increases the number of units per intake. In these cases, it is important to provide easily and explicit instructions to the patient, employing pictures and pictograms on the patient kit to increase compliance [14]. In both cases, neither the patient nor the clinician would know which active drug was being administered. Table 9 defines common types of blinding used in clinical studies.

Table 9. Common Types of Blinding used in Clinical Studies. Adapted from Jeatran and Clark (1998) [165]

Open Label	No blinding is used; participants know the identity of the treatment
Single Blind	Only the patient is blinded
Double Blind	The patient and clinical investigator are blinded
Triple Blind	The patient, the clinical investigator, and the sponsor are blinded
Double Dummy	Using active and placebo form of both the study drug and the positive control drug or drugs to blind a study
Third Party Blinding	Use of an unblended third party (e.g. pharmacist or nurse) to dispense drugs to blind a study

There are many blinding options available today (table 10), i.e., deprinting, mill & fill, remove markings, overcoat, similar-looking products, etc. [166], but over-encapsulation method still seems to be the most popular method. Perhaps over-encapsulation is not the least complex, but it is the most commonly chosen option for blinding clinical supplies [167, 168]. Indeed, the over-encapsulation nowadays is a state-of-the-art technique for blinding of comparators, which consists in the over-encapsulation into hard gelatine capsules [163].

Table 10. Techniques and Considerations for Blinding of Drug Products. Adapted from Carney *et al* (1995) [166]

Type of original oral dosage form	Technique	Considerations
Tablet	Removal of markings	Time consuming and manually intensive; Still must match size/shape/color of tablet; Process may alter release properties of a film coating.
	Over-encapsulation	Time consuming and manually intensive; Patients may open capsule and discover original dosage form.
	Grinding and re-tableting	Properties of new dosage form may be different from original form, affecting patient response; Complexity in developing new formulations.
	Grinding and encapsulating	Properties of new dosage form may be different from original form, potentially affecting patient response; Assuring blend uniformity of grinded dosage form while encapsulating; Manually intensive, unless automated encapsulating equipment is available.
	Tablet overcoating	Tablets are developed to match original dosage form; Preserves original dosage form; Pharmaceutical testing (i.e. dissolution) can help verify similarity to original dosage; Cannot be used for embossed tablets.
Capsules	Removal of markings	Time consuming and manually intensive; Still must match size/shape/color of capsule.
	Over-encapsulating	Same as above tablets.
	Grinding and re-encapsulating	Capsule shells from original dosage form may be visible in manufactured product.
	Removing ingredients from capsule shells and encapsulating	Manually intensive; Time consuming; Most capsules are difficult to open.
Oral solutions	Matching solution without active ingredient	Taste and odor may be unique to active ingredient; Discoloration of active ingredient over time may occur and cause unblinding.

Over-encapsulation corresponds to basically hiding another dosage form, tablet or capsule, inside a capsule shell. It is important to select the appropriate components that will be needed to support the over-encapsulation of the tablet or capsule unit. Once the unit has been identified, the first thing to determine is what size capsule shell will needed to be utilized to properly blind each unit. Although it is not completely necessary, it is recommended that the unit that is being encapsulated does not protrude above the body of the capsule shell when inserted. The most common challenges of over-encapsulation are its possible effect on dissolution, disintegration, and bioequivalence in clinical trials, as well as the interaction between the backfill and the gelatin capsules [167, 168]. However, according to the GMP regulations, study sponsors must provide data demonstrating that over-encapsulation will not alter product quality. So, the use of backfill requires additional compatibility and pre-formulation studies. The dissolution profile of an over-encapsulated product largely depends on the properties of the API, the characteristics of the over-encapsulated unit, and the backfill [168, 169]. According to Faust (1999), the choice of backfill may affect dissolution of an over-encapsulated comparator product [170]. Lab tests showed that the dissolution of the over-encapsulated drug has a time interval of about plus 5-10 minutes in comparison to the original product [163].

3.1.3.3. Sourcing and Development of Comparators

As mentioned above, comparative effectiveness studies for new drugs are multicenter, and trials can include an active comparator, despite of comparison against a placebo, is generally required by regulatory authorities, mainly when a pharmaceutical company seeks to claim superiority or non-inferiority versus the competitor drug in its marketing materials [164, 171].

The process of sourcing and supplying comparator drugs for global clinical trials represents a number of obstacles and potential pitfalls for the trial sponsor [171, 172]:

- Inability to obtain the necessary pedigree and product documentation for the comparator;
- Lack of supply chain security with possible introduction of counterfeit comparators;

- Delays in resupply throughout the course of the trial.

Drug developers must have a comprehensive view of what is involved in the process and formulate a strategic approach to best support their trials [164, 171].
In order to minimize the risk of delayed or interrupted clinical trials and the financial implications that can result from it, a comparator sourcing and supply strategy should include a number of elements, such as [164, 171]:

- A thorough understanding of global regulation governing sourcing;
- Proactive demand planning that can rapidly predict changes in the quantity of comparator needed, duo to patient enrollment or unexpected trial changes.

One of the simplest ways to reduce risk during the comparator sourcing process is to start planning early, typically during the protocol design phase [171], because the ultimate goal of comparator sourcing is to get the right drug, to the site, at the right time. Indeed, the efficient management and movement of trial supplies, including comparator drugs, is an important factor in the success of any trial [164].
According to Dutta [164] and Goodson *et al* [157], sourcing directly from a manufacturer enables access to large, single lots of the comparator with maximum shelf-life, and specific batch numbers when possible. Comparators with a maximum shelf-life provides time for characterization, demonstration of bioequivalence, relabeling and repackaging, if needed, while minimizing the frequency of costly resupply [164].
Regarding to development of comparators, it is very similar to NCE development, but is somewhat abbreviated on a compressed time line [157]. The development should start with an assessment of potential blinding techniques. So, the formulator should strive to minimize the potential risk of producing a blinded dosage form that does not perform exactly as the initial comparator product, and choose the blinding technique that requires least amount of manipulation, maximizes the potential to manufacture a bioequivalent and stable product, and adequately blinds the clinical dosage form. However, if comparator dosage form must be manipulated (e.g. milled, over-encapsulated, etc.), pre-formulation studies may have to be performed, including dissolution rate, moisture, particle size, hardness, physical size and weight (for solid dosage forms) and accelerated excipient

compatibly studies. Also, formulation studies have to be performed, including short-term stability, excipient effects, comparative dissolution for solid dosage forms, and, depending on the degree of manipulation, *in vivo* bioequivalence testing [157].

3.2. Packaging

The submission of an investigational new drug must include detailed information concerning the packaging component [173].

IMPs are normally packed in an individual way for each subject included in the clinical trial. The number of units to be packed should be specified prior to the start of the packaging operations, including units necessary for carrying out quality control and any retention samples to be kept [11].

During packaging of IMP, it may be necessary to handle different products on the same packaging line at the same time, so the risk of product mix must be minimized by using appropriate procedures, specialised equipment as appropriate and relevant staff training [11].

The selected packaging must be appropriate to enhance stability and compliance. The selection of a container depends not only on the physicochemical properties of the drug and the intended use of the dosage form, but also the environmental conditions to which the product will be exposed and the characteristics of the formulation [14, 174]. Packaging materials should not interact physically or chemically with the preparation [39, 173, 174], and container selection should take into account characteristics such as visibility, strength, rigidity, moisture protection, ease of reclosure, and economy of packaging [174]. Waterman *et al*, also considers that package selection can be based on other considerations, such as cost [175].

Several types of packaging will be employed: the primary packaging (direct contact with the product) and the secondary packaging (such as carton). The majority of packaging materials are in some degree permeable to moisture and the type of closure employed, such as plastic and screw fittings, may also permit the ingress of moisture. Thus, specialized packs, using low permeability materials, may be required [14]. Temperature fluctuations can lead to condensation of moisture on the product, and with liquids, this can lead to

microbiological contamination. But light, oxygen and mechanical damage is also a challenge in packaging [14, 39, 173, 176].

Also, the type of study, and the frequency and duration of the dosing regimen will influence the package selection. For example, for a small preliminary study it might be preferable to simply manually package into bottles of unit dose systems [173]. But, in general, the package should be inert to the product within, be adapted to the study, allow for the easiest and most accurate accumulation of clinical data, and offer the greatest assurance of patient and site compliance [14, 173].

In accordance with the methods of use and administration of medicinal products, packaging materials, closures and containers can vary with a wide variety of different requirements. To ensure the efficacy of a product during its total shelf-life, pharmaceuticals must be regarded as a combination of the medicinal product itself and the packaging.

Only the most commonly used packaging materials and containers are described in table 11.

Table 11. Packaging Materials and Containers. Adapted from WHO Annex 9 (2002) [176]

Packaging Materials and Closures	Advantages	Examples
Glass	First choice; Glass can be tested for light transmission and hydrolytic resistance.	Bottles for tablets, injection syringes for unit- or multi-dose administration
Plastic	Unbreakable; Collapsible.	Bags for parenteral solutions.
Metal (aluminium and stain-less steel are the metals of choice for both primary and secondary packaging for medicinal products)	Provide excellent tamper-evident containers; Strong; Impermeable to gases and shatterproof; The ideal packaging material for pressurized containers.	Tubes, packs made from foil or blisters.

The quickest route to a package product is to use bottles, but in the case of complicated dosing regimens, compliance may be compromised [173]. On the other hand, blister cards allow that correct dosage is taken at the proper time, which directions indicate for used printed on the card. Of course, this assumes that the patient remembers to take the medication [173, 177].

According to Pilchik [177], in addition to help patients following drug regimens, blister packs can also protect drugs over a long shelf-life, and are portable. Advocates of blister packaging cite some advantages of blister packaging:

- Product integrity;
- Product protection;
- Tamper evidence;
- Reduced possibility of accidental misuse;
- Patient compliance.

Aluminum foil is the usual choice of backing for the blister unit [173]. To ensure functional properties of blister packages, the quality of starting materials, as well as the sealing process validity in the packaging process are most important. In particular, cold form blisters have to meet high standards, as they are commonly used to protect moisture sensitive products. The investigations of Muhlfeld *et al*, using different grammages of heat seal lacquer, showed that the influence of heat seal lacquer grammage on the quality of blister packages seems commonly overestimated [178].

Regarding to closures, they are used for the purpose of covering drug containers after the filling process, and should be as inert as possible. They should not give rise to undesired interactions between the contents and the outside environment, and should provide a complete seal. Besides their protective function, closures must also allow the easy and safe administration of the drug [176].

Depending on the type of container, closures may have different shapes and sizes, such as stoppers for infusion or injection bottles or plungers for prefilled syringes. A special design of stopper may also be required for some pharmaceutical production processes such as lyophilization [176].

3.2.1. Stability Testing in Clinical Packaging

In the selection of a package suitable for a clinical trial falls into the product evaluation on the following characteristics [173]:

- Sensitive to moisture (solid dosage forms);
- Light sensitive (solid and liquid dosage forms).

It is the research pharmacist's duty to stress-test the drugs in storage using factorial combinations of [39]:

- Low and high temperatures;
- Low and high moisture;
- Exceeding the labelled drug shelf-life;
- Exposure to bright and subdued light (in some case clear and amber glass bottles).

The needs of the product must be defined, and the packaging must reflect the needs. It should avoid the mistake of package first and discover later that the package failed to protect the product. Component selection should be based on data, not on expediency [173]. Table 12 describes some of the products needs of sensitive products and the solutions for packaging them.

Table 12. Solutions for Packaging Sensitive Products

Product is very sensitive to moisture	Desiccants may have to be added to the container or suitable film select that will afford moisture protection [154, 173].
Product is light sensitive	Some sort of light protection is required, like amber film, opaque blisters, opaque hard gelatin capsule shells [154, 173].

3.3. Labelling

Labelling is an important and integral part of the approval of an IMP in clinical trials [173], and it should comply with the requirements of Directive 2003/94/EC [11, 179].

The label has to be permanently attached to the container. The challenge is increased in multinational trials, in which the necessity arises to give information in several languages (multi-lingual trials).

The compliance with the labelling requirements is important for all clinical trials during drug development, since non-compliance may cause problems during the later approval process for the marketing authorisation application because this may be regarded as non-compliance with Good Clinical Practice (GCP) [179].

The regulatory affairs manager is responsible for the labelling of a medicinal product, and, therefore it should coordinate the decision process for the labelling in clinical trials, also because this labelling is part of the basis for the future labeling [179].

The basic information on the label should provide the patient's name, study phase, trial reference code, batch number, pharmaceutical dosage form, route of administration, quantity of dosage units, directions for use, any special warning or storage requirements, expiry date and investigator's name and address, along with an indication that the drug are "for clinical trial only use" and should "keep out of reach of children" [11, 14, 179, 180]. Furthermore, the sponsoring company's name and address should also appear [14].

CHAPTER 4

Handling Clinical Trial Supplies

The clinical trial supply chain is an enormously complex process. Ensuring that supplies arrive at the trial sites on time and in good condition is a challenge that clinical trial supply chain managers face in global distribution. Effective distribution requires a knowledge of regulations in trial countries, qualified suppliers and storage infrastructure, and control of supply temperature and conditions for the duration of delivery [181].

The clinical trial material supply chain comprises the planning and scheduling of all transactions, operations and organizations during a trial, beginning with API manufacturing, followed by drug manufacturing and distribution to the clinical sites, and ending with dispensing the drugs to patients at each clinical sites [182, 183]. Figure 12 represents schematically the typical clinical trial supply chain.

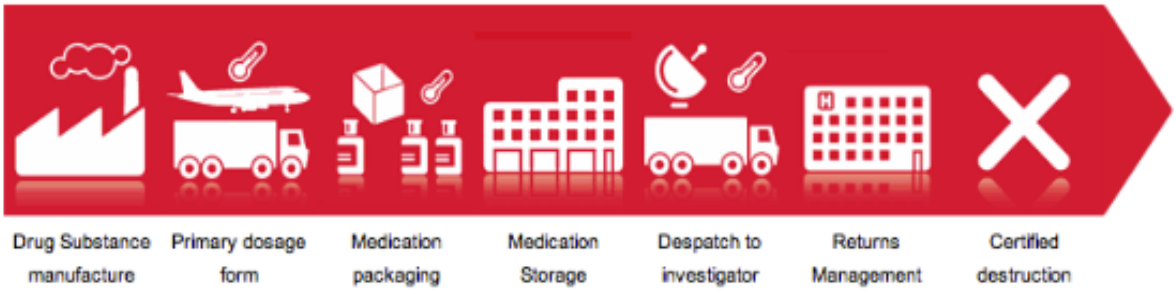

Figure 12. The Traditional Clinical Trial Supply Chain Model. Adapted from Klim (2009) [184]

Traditionally, the pharmaceutical industry uses batch processes in the manufacture of pharmaceutical products. Since these batch facilities are usually shared across various products, especially for the quantities needed for clinical trial, it is necessary to decide on the order and timing of the products to be produced. These decisions can have a large economic impact on the company at the clinical trials stage, because missing the delivery of trial dosage to patients can significantly delay completion of the trial, and consequently, delay the time to market, which in turn can mean increased costs [182, 183].

The key technical challenge in managing a clinical trial materials supply chain are to meet the needs from clinical sites, minimizing delays but also oversupply, and to ensure that unused materials are not re-routed to others clinical sites [182, 183].

The distribution of clinical trial supplies presents several challenges that can be differently identified by the involved staff:

- Supply-chain managers point several problems in clinical operations [185]:
  - Failure by clinical operations to provide accurate patient-enrollment forecasts;
  - Lack of resources and capacity in clinical operations and distribution;
  - Lack of processes and distribution lanes for new country distribution;
  - Poor clinical visibility and interactive-voice-response-system (IVRS) performance.
- Clinical operations personnel point weaknesses in supply-chain [185]:
  - Increased globalization of clinical trials and the resulting problems in rest-of-world distribution management that cause delays in receiving clinical trial material (CTM) on time. These problems involve delays in the customs-clearance processes, a lack of distribution capacity, and poorly established distribution lanes.

Other concerns in the supply-chain include poor planning processes in clinical contract manufacturing that can cause delays, as well as long lead times for comparator sourcing. Another cause for delay in CTM supply relates to securing internal approvals for sourcing and contract-manufacturing arrangements. “A lack of a framework and direction on approval rules and policies can cause undue delays,” says Handfield and McCormack [186]. Defining more clearly and streamlining the approval process through improved documentation, the use of contract-manufacturing and sourcing approval coordinators, and automation of the approval process, are some actions to address these problems.

4.1. Product Handling Parties in the Clinical Trial

The clinical trial system requires cooperation of diverse groups, including personnel from the pharmaceutical company's medical department, biostatisticians, investigators, trial subjects, representatives of national and international regulatory organizations, manufacturing personnel, packaging personnel, and sometimes representatives from contract research organizations (CROs) and other third-party vendors [187].

All personnel involved in distribution activities should be trained and qualified, based on written standard operating procedures (SOPs). Personnel should receive initial and continous training relevant to their tasks, in order to ensure quality of the product [188].

4.1.1. Sponsor

The sponsor is generally a company, institution or organization which takes responsibility for the initiation, management and financing of the clinical trial [11].

4.1.2. Investigator

Clinical investigators are composed by physicians who provide the drug to the patient, and they are sometimes also responsible for reordering new drug supplies [187]. They are responsible for the conduct of the clinical trial at a trial site, and the responsible leader of a team of individuals involved in the conduct of trial [11]. However, they may have difficulty keeping track of various SPOs, storage conditions, minimum inventories, and other rules related to a range of studies and products, and, therefore, pharmacists and study coordinators plays also an important task in help handling the IMP. Investigator responsibilities include:

- Subject recruitment;
- Medical care of subjects;
- Collection of subjects' informed consent forms;
- Communication with Institutional Review Board (IRB) and Independent Ethics

Committee (IEC);

- Protocol compliance;
- Investigational medicinal products;
- Randomization and blinding procedures;
- Data reporting;
- Safety reporting.

4.1.3. Pharmacists

Investigational sites have, usually, one or two dedicated clinical trial pharmacist, despite of the current constraints in health service funding. The pharmacy team should often meet with the investigator team during the study [189].

The pharmacist should be responsible of store the IMP, separately from normal pharmacy stock, in an area with restricted access. IMPs that are returned by patients or have expired must be stored separately from unused IMPs. Also, the pharmacy staff must undertake regular temperature monitoring of IMPs storage facilities, and these records archived [190]. A complete summary of drug dispensing, as well as records of procedures and responsibilities should be safe with the pharmacist [191].

In summary, the tasks of the pharmacist during a clinical trial are [190, 192, 193]:

- Receipt and recording of the safe delivery of IMPs;
- Safe handling and storage of IMPs;
- Preparation and dispensing of IMPs in accordance with professional standards (including dispensing against an appropriate prescription, maintaining drug accountability records and ensuring that all IMPs are labelled with the appropriate pharmacy label);
- Return of unused IMPs;
- Reconciliation of IMPs;
- Maintaining a pharmacy study file;
- Training of clinical trial pharmacy staff.

4.1.4. Study Coordinator

The Clinical Research Coordinator (CRC) is a specialized research professional working with and under the direction of the clinical principal investigator. While the principal investigator is primarily responsible for the overall design, conduct, and management of the clinical trial, the CRC supports, facilitates and coordinates the daily clinical trial activities and plays a critical role in the conduct of the study. By performing these duties, the CRC works with the investigator and sponsor to support and provide guidance on the administration of the compliance, financial, personnel and other related aspects of the clinical study [194].

The study coordinator responsibilities include [192]:

- Patient recruitment activities, supporting the investigator;
- Completing case report form;
- Transmitting study data;
- Scheduling patient visits;
- Meeting with principal investigator;
- Meeting with study monitors;
- Shipping samples to laboratories;
- Maintaining inventory and accountability of the investigational product;
- Closing out the study;
- Participating in ongoing training.

It is important to highlight that the CRC, and also the principal investigator, works with various sponsors or CROs at the same time. These different sponsors or CROs communicate at different level of expectations regarding source documentation. If the site is not experienced enough, and those professionals do not have a standard procedure to follow, they may get confused with variations in guidance they receive. This may negatively impact the quality of data [195].

4.1.5. Study Monitor

Accurate drug accountability should be ensured by study monitor, during routine visits with the aim to assess the site's drug preparation and dispensing procedures, as well as subject compliance, and proper drug storage [196].

Monitors should confirm that all supplies are accounted for and returned or destroyed, and any discrepancies should be investigated and resolved before site closure. Monitors can identify inappropriate practices by reviewing drug accountability documentation, and retrain site staff as needed. Proper review of drug records can find poor subject compliance with drug administration, incorrect supplies at the site, incorrect drug preparation and randomization, and potential unblinding of subjects. Upon noting problems, monitors should immediately implement corrective action plans to prevent future occurrences, since problems detected early are more easily resolved [197].

Drug accountability documentation (that are part of regulatory documents) should be simple and useful, and it allows [196]:

- Document the handling of the study drug from receipt to dispensing and return;
- Display inventory, lot numbers, dose sizes, stock quantities, and expiration dates;
- Include shipping invoices, confirmation of receipt, condition upon receipt, and the information of when drug was received, dispensed, and returned to the sponsor;
- Help verify patient clinical records and detect possible lot variations;
- Help verify patient case report form and detect any incompliance;
- Help identify patients who may have received the drug, as well as the quantities that they may still have in their possession;
- Support the validity of study data and conclusions drawn from those data.

4.1.6. Patients

Trial subjects are responsible for taking the IMP, and they present multiple challenges to the packaging group and clinical supply group. For example, subjects might misplace their drug supplies or not take the product as labelled [187].

When supplies are dispensed to subjects for self-administration (ambulatory), compliance should be ensured, using, for example, diary cards. Furthermore, in order to assess compliance, investigator should ask unused supplies and empty containers.
In fact, all data obtained from the patient are relevant for the assessment of clinical trial results, and patients should be instructed to meet all the criteria for data registration.
Also, all patients who participated in clinical trials should be followed until the end of it. Loss to follow up can change the results and compromising the study.

4.1.7. Contract Research Organizations

CRO's are organizations contracted by the pharmaceutical company to support pharmaceutical development, preclinical research, clinical research, clinical trials management (manufacturing, packaging, labelling, and distribution clinical materials), as well as pharmacovigilance activities [180, 187].

4.2. Distribution of Investigational Medicinal Product

Figure 13 shows an example of supply chain. First, the API and drug product are manufactured in a silo type organization with departments that have a science focus on the development of API and drug product. Manufacturing process is obviously a key deliverable from those departments. Comparator drug manufacturing can be defined as a normal drug product manufacturer, but dynamics are different, typically sourced via intermediate entities and it drives the study supply costs significantly. The packaging unit for IMP is linked with blinding aspects of the trial [198]. Second, figure 13 also shows that IMP is transferred to a distribution network, establishing a proper supply chain for each study. The distribution ends at the patient visit in clinical sites, potentially managed with interactive response technology from CROs or specific interactive responsive technology service providers [198].

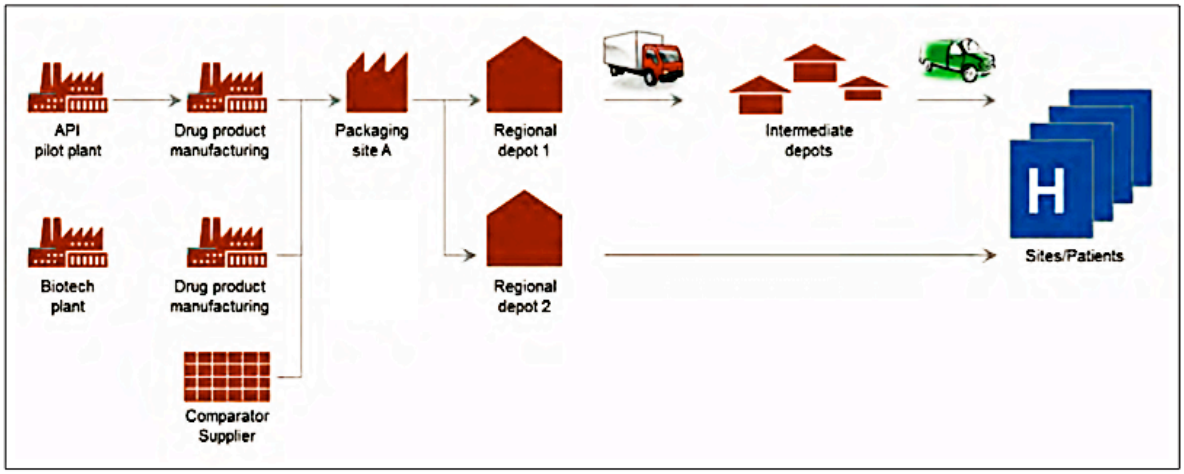

Figure 13. The R&D Supply Chain. Adapted from Bielmeier & Crauwels (2012) [198]

Regardless if the activity is internally executed or outsourced, sponsors and contractors need to overcome many operational challenges, such as forecasting, planning, manufacturing, warehousing and distributing drug product [198].

4.2.1. Warehouses/Depots

For large global studies, depots are often an integral part of delivering supplies in a timely, cost-effective manner.

Depots should have drug storage facilities compliant with GMP, with controlled room temperature and cold-chain storage. Often depots are established in areas where there are lengthy customs clearance and import license application processes, complex import requirements, and long shipment times due to distance [181].

In countries such as Canada and Australia, the deposit delivery is unnecessary, since the customs procedures are simplified. But in other countries, such as Argentina, Russia, China and India, a deposit is required because of sheer distance, and are countries that require considerable time to clean materials [181].

Depots are also advantageous when clinical centers require clinical supplies within a short time, and when there are large numbers of patients and sites in one area, reducing the risk and cost of cold storage shipments [181].

Another important factor in the use a depot is cost, considering that it is far less costly to deliver one or two bulk shipments to a depot, than repeated shipments directly to sites, which mean also an increase risk. But, it is also important, find out if depot has the capability to recover unused supply from the site and ship it back [181].

The requests for serialized kits[3], such as depicted in figure 14, needs to be a highly automated process between depot warehouse and order management, in order to ensure compliance, avoid errors, and control the costs [198]. Once clinical trial material (CTM) is prepared into patient kits, shipment to clinical sites can be dictated by patient enrollment. For multicenter studies, initial supplies can be used to open a clinical site where resupply frequency is dependent on actual patient enrollment at any given site. During shipment, IMPs should be protected against exposure to undesirable environmental conditions, maintaining, for example, freezing or refrigerated conditions to ensure product integrity, especially in cases of biological products shipment [180].

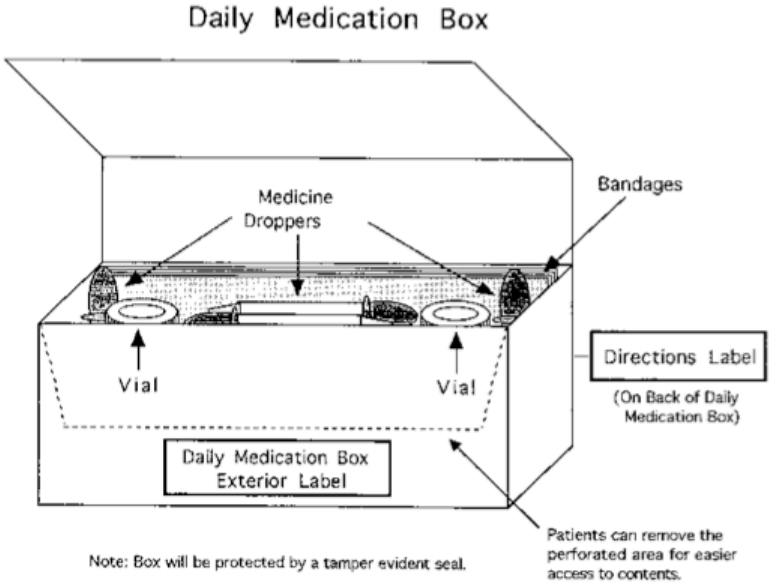

Figure 14. Content of Patient Kit. Adapted from Bernstein (2002) [154, 180]

[3] Assembly of clinical trial material into patient kits is known as secondary packaging, since the actual drug product is not exposed to environmental conditions during this operation. The use of patient kits facilitates dispensing by the clinical site, usage by the patient and returns goods accountability by the clinical monitor.

Sponsor pipeline products are becoming increasingly cold chain with the influx of biomolecules that needs a strict temperature measurement method. But such methods are becoming increasingly costly, often representing a burden for the launch of biomolecules [198].

4.2.2. Intermediate Storage

Regarding to intermediate storage, a more common approach has been defended in last few years, in order to eliminate the intermediate storage lead time at local or country specific depots: direct to site shipment from regional hubs [198]. In reality, this is one of the most commonly employed strategies. Clinical supplies are stored within a central depot and are exported to investigator sites in various countries as required [199].

But, many companies successfully use a local distribution depot within required countries. However, this concept requires application of multiple import licenses, and allows the depot to act as a repository for clinical trial supplies [199].

The next table shows the advantages and disadvantages of direct to site distribution, and distribution to local depots.

Table 13. Advantages and Disadvantages of Direct to Site Distribution and Local Depots Distribution. Based on Shannon (1999) [199]

Direct to site distribution		Distribution to local depots	
Pros	**Cons**	**Pros**	**Cons**
Material may be labeled with multiple languages	Direct to site shipment is not possible for some countries	Reduced transit times to investigator sites	Management of multiple local depots
No pre-allocation of supplies to specific countries, allowing maximum flexibility	Frequent, low volume international shipment	Single customs clearance per depot	Wastage of supplies through initial overstocking of depots
Reduced manufacturing overages required, leading to reduced costs	Depending on the countries selected, multiple import license applications may be required	Reduced shipping costs – one large international shipment to a depot, followed by multiple domestic shipments will be less expensive that multiple international shipments	Reduced visibility of supplies at depots – inventory reports may not be as readily available
Greater control of supplies reducing the amount of unused medication		Readily available local knowledge and expertise	Medication must be pre-allocated to countries
			Greater risk of loss should there be any deviations associated with the initial depot shipment

4.2.3. Import Licenses

Emerging markets, such as Asia, Central and Eastern Europe, and Central and Latin America, offer an opportunity to reduce R&D time and costs if managed appropriately. To conduct these trials successfully it is critical to appreciate the complexities of shipping to emerging countries and ensure that any potential benefits are not lost through a lack of understanding or poor planning [199, 200].

One of the largest hurdles to pharmaceutical companies conducting trials in emerging countries is the import requirements [201].

Clinical trial supplies should be available at clinical sites when are required, to ensure the success of any study. The import of clinical supply materials is highly regulated and needs to comply with country specific regulations. Failure to comply with import regulations could mean the end of a study, due to, for example, the refusal of a carrier for transporting supplies. Trials occurring in emerging markets present some additional challenges, such as [199]:

- Political instability;
- Poor transport infrastructure;
- Poor training of staff;
- Time required to obtain appropriate import licenses.

Obtaining an import license for shipment can be a time consuming process, where is necessary an accurate completion of documentation. Often inexperience in import license application or lack of information during preparation of the data to be submitted, leads to delays in the initial application, as well as customs delays, because of the use of incorrect information. In most cases an entirely new import license application may be required. Country specific regulations should be fully understood and complied to maintain a fluid supply chain. In addition, it is important to have knowledge of public holidays and regulatory agency “shut downs”, because the lack of this information could also face considerable delays in an import license application, being not processed promptly [199].

Generally the CRO will arrange the import licenses and permits for the clinical trial, and the supplies company will produce the customs invoices [201].

4.2.4. Control of Transport Conditions

4.2.4.1. Shipment Logistics

Shipping volumes of pharmaceutical drugs need logistics specialists, the national or international transport carriers, freight forwarders, and brokers that make it all happen.
Depending upon how the product is classified, in terms of International Air Transport Association regulations, different requirements would apply. Additionally, the importing country may also impose their requirements. It makes sense to choose a customs broker with offices located in the country closest to the shipping source or distribution point, with expertise in the regulations of that country. Depending upon the size of the shipment, the clinical supplies unit may need to contract a courier to transport the CTMs from the company to the investigator or depot distribution site. The advantage is that the properly qualified courier company will know all of the key requirements of importation and exportation to assure delivery at the clinical site [202].
Although it is important to produce quality CTM, it is also important to provide CTM worldwide, safely and in a timely manner. Use of various services like an express mail courier (i.e., DHL and FedEx), freight forwarders, shipping agents, and truly specialized couriers (i.e., World Courier) should be chosen based upon the product that needs to be shipped, its size and weight of the shipment [203]. Their ability to know and satisfy the requirements in the receiving country (import and export requirements) is essential. This should include, but not be limited, to the following services [202]:

- Refreshing cold packs or dry ice;
- Expedited delivery of the product;
- Ability to transport large quantities;
- Ability to transport hazardous or flammable products;
- Knowing the requirements in both the importing and exporting countries;
- Ability for rapid customs clearance;
- Obtaining required documentation;
- Paying duties or customs charges;
- Having a general knowledge of the infrastructure within the importing country.

The vehicles and equipment used to distribute clinical supplies should prevent exposure to conditions that could affect their stability and packaging integrity, as well as any kind of contamination. So, the vehicles, containers and equipment should be kept clean and dry, and free from accumulated waste [188]. Written procedures and records for cleaning should be in place to ensure the quality of the IMP, but also management should approve the agents used for the cleaning [188].

One of the trends happening in the industry is that the traditionally regional transport companies are building more extensive networks in order to maximize their business. Another recent trend among logistics specialists has been a shying away from airfreight [203].

4.2.4.2. Temperature Control During Transport – Good Cold Chain Management Practice

The distribution of temperature-sensitive drugs represents an increasingly important process of the global pharmaceutical supply chain.

Of the 427 billion € of pharmaceutical product sold world wide in 2005, more than 10% were biopharmaceuticals, which grew faster than the traditional pharmaceutical market [204].

Clinical trials run on a global scale and in some cases in markets with less than ideal logistics infrastructures, creating a challenging distribution environment because of shipping large volumes of refrigerated patient kits worldwide, while maintaining and documenting appropriate environmental conditions. Moreover, the globalization of clinical trials needs specialty couriers and contracted depots to improve the performance of the supply chain and build a more robust clinical trial distribution process [204].

The impact of cold chain failure on clinical trial material may lead to the following risks [205]:

- The patient could be administered an unsafe product;
- Lack of compliance with global regulatory and standards-based requirements can increase liability;

- Thermal variability can lead to inconsistency of results between and within batches;
- The shipment can be rejected by the Quality Department therefore leading to costly delays.

The debate between appropriate shipping and storage temperatures requires supporting data and a transportation control strategy [206]. John Taylor and Ian Holloway, from the Medicines and Healthcare Products Regulatory Agency in United Kingdom, state in an article: "the need for control is even greater for clinical trial materials because, in early phase studies, the stability of the material may not have been fully established. The redeployment of CTM to different trial sites can present additional risks to these materials" [207].

Four primary regulatory trend have been identified [204]:

- Accountability for the cold chain should be shared across all supply chain partners;
- Increased oversight, management, and control of environment conditions across the entire supply chain;
- Increased importance of the temperature control and monitoring;
- Heightened priority of patient safety – with focus on product quality.

The common goal of all partners should be common: ensuring that each patient and site is supplied with the correct medication, at the right time, and in the right condition.

4.3. Stock Management

4.3.1. Site Activities

IMP accountability is the process for accounting and documenting the use of the IMP, keeping in mind compliance and potential loss of the medication [195, 208]. It includes:

- Receipt and inventory of investigational drugs;
- Storage of IMPs, including a limited-access area and proper temperature conditions;
- Dispensing of IMP;
- Return of IMP;
- Maintain accurate and complete drug inventory records, including the recording of dates, lot numbers, date the drug is received, and date dispensed to a subject or returned to the sponsor.

The research pharmacist must properly store and dispense all IMPs and maintain accurate dispensing and inventory records.

4.3.1.1. Receipt

The receipt of each shipment of study medication or device should be confirmed in writing by the investigator or pharmacist (or other authorized personnel), who will be instructed to return a completed "acknowledgement of receipt form" immediately [209]. After receipt, supplies must be checked against any shipment form to ensure that IMPs received corresponds with what was requested. The following checks should be made [208]:

- Ensure supplies are correctly addressed;
- Ensure all packaging intact;
- Ensure that the quantity, batch numbers, name of manufacturer, correspond with shipment form.

If any problems are detected, such as missing or broken items, defects in labelling, or inappropriate temperature control, sponsor or CRO should be immediately informed. [209]. Any discrepancy is a big problem, not only because of the regulatory violation, but because it potentially endangers the integrity of the study [208].
Once received the investigational products from sponsor, the pharmacist or principal investigator must send to the sponsor a filled clinical supplies acknowledgement form

(table 14) through mail or courier systems [208].

Table 14. Clinical Supplies Acknowledgement Form. Adapted from Krishna *et al* (2012) [208]

Clinical Supplies Acknowledgement Form	
Study No:	Received by:
Received date/Time:	Received through (by Courier/by Post):
Storage Condition:	Packaging condition:
Any special condition:	Number of container received:
Total quantity in test container:	Total quantity in reference container:
Any discrepancy:	

The exact date of receipt of the clinical supplies should be promptly recorded, so that the monitor can determine that the supplies were secure and correctly stored during the entire period of shipment [209].

After the clinical study supplies have been sent to the study site, the monitor must verify, as soon as possible, that the supplies have arrived satisfactorily. Monitor should check the condition of the supplies before subjects receive medication. The study monitor will verify that the amount shipped matches the amount acknowledge as received, and, if there is a lack of reconciliation, recruitment may be delayed until the situation is resolved [209].

Failure to check the receipt of IMP into pharmacy stores may result in an incorrect audit trial of drugs received, incorrect drug accountability or the drugs received being stored in incorrect temperature conditions. This could result in IMP being wasted and cancellation of patient recruitment.

4.3.1.2. Storage

Evidence of careful control at the study site is imperative, but is difficult to standardize the situation across many study sites and many countries [209].

According to the ICH Guideline for GCP, after checking the supplies received at site, they should be stored in appropriate conditions, as specified by the sponsor [210]. The drug storage area should be secure. It should be locked where appropriate and access to the supplies limited where possible to investigators and research nurse.
The study monitor must be trained to check correct storage and documentation, and ensure that all site personnel are fully trained and informed [209].
During the process of pharmacy, the required storage conditions for the IMP should be ascertained by reference to [211]:

- The Investigator's Brochure or Summary of Product Characteristics;
- The Protocol.

The main concern for appropriate environmental conditions is usually temperature requirements, especially with biologic supplies that are usually to be kept refrigerated between 4-8ºC. But light, humidity and room temperature are also important, because such terms could have different meanings in different counties [209].
There are also two quarantine storage areas for IMP, one for refrigerated IMP (2ºC to 8ºC) and another at room temperature (15ºC to 25ºC), which should be used to store IMP if the product has expired, or is awaiting certificate of release, or has been subject to a temperature excursion. Ambient and refrigerated supplies returned by the patient must be stored in a box labeled "Clinical Trials Returns". Once they have been reconciled and logged in the relevant pharmacy clinical trial file, they will be stored in the clinical trials office, in a locked cupboard separated from normal hospital stock [211]. A record of storage temperature conditions should be maintained for each location where current supplies of IMP are stored [211]. At each monitoring visit, the monitor will ensure that the correct procedures are being followed [209].

4.3.1.3. Dispensing

All investigational drugs should be signed out on Investigational Drug Accountability Form by the pharmacist who dispensing the drug (table 15). Each separate drug dispensing form should be used for each protocol [208].

An investigational or study drug may be dispensed only upon receipt of a written order by the principal investigator [212]. However, there are additional requirements for dispensing investigational drugs, which include: verification of protocol approval and informed consent, record-keeping, preparation or packaging of final product, labeling of dispensed product, and disposal of unused or partially used medications [213].

All relevant information and documents for proper and safe drug dispensing must be widely available. The pharmacy staff needs to know not only how to prepare and dispense the drug but also how to complete dispensing records and maintain inventory, in order to successfully adhere to the clinical drug protocol. Pharmacists who dispense IMPs must be familiar with the drug information and study design, in order to assist the clinical staff with management patient [212].

Table 15. Drug Dispensing Form. Adapted from Krishna *et al* (2012) [208]

Name of the Investigational Product (Test/Reference):							
Batch Number:							
Expiry Date:							
Period	Date	Line clearance Yes/No	Initial balance of drugs	No. of units dispensed	No. of units dosed	Remaining Units	Allotment of subjects as Per Randomization Schedule
Dispensed by:				Checked by:			

There are situations relatively uncommon, in which the investigator carries out the storage and dispensing of the IMP, such as when the immediate administration of or facile accessibility to the IMP is necessary to ensure adherence to trial methodology [213]. This situation requires policies and procedures successfully implemented to ensure that the pharmacy maintains some oversight of investigational drugs [213].

Since the use of drug in clinical trials within the institutional setting must be done in accordance with a number of regulations, guidance, and standards, including GCP guidelines, local institutions policies and procedures, and the study protocol itself, in a

recent article, Siden *et al* [212] describes a tool that can be implemented by pharmacists to prepare instructions for dispensing, inventory management, and accountability of IMPs in a consistent, concise, and organized manner. The template is divided into three major sections [212]:

- Dispensing instructions (includes aspects related to the correct distribution of the drug, including dose calculation, calculation worksheets, drug preparation and dispensing procedure, and documentation of drug dispensing);
- Study design and drug information (provides background information described in the protocol and the investigator's brochure, including pharmacokinetics, adverse effects, and concomitant drug restrictions of the investigational drug);
- Study maintenance information (included instructions on drug ordering and disposition and information on the sponsor as it relates to sponsor visits and audits).

During an audit, a random sample of dispensing records and inventory log forms are inspected [213]. Dispensing by the pharmacist is a crucial step in the handling of the investigational drug, since the most common pitfalls identified during sponsor audits are [195]:

- No documentation that oral drug was provided to subject and/or returned by subject with pill count;
- Poor documentation regarding drug diaries;
- Poor prescription practices.

Since source documentation related findings are the most commonly cited during inspections and audits [214], the clinical site should develop SOPs for good documentation [215]. The SOP should be shared with the sponsor/CRO and agreed before the start of the trial. SOP should also address aspects including consenting process, verifying eligibility, use of right tools such as diaries, source document worksheets, copies of prescriptions, etc; ways to avoid multiple records, and in case of multiple records, should define the source for the study, method of corrections, review of safety labs and other reports. Documented procedure at site level should encompass management, maintenance, archival and retrieval of source documentation, in order to have measures for continuous improvement and

maintaining high-quality data, because is extremely important that sites develop process for quality control [195].

Since drug accountability records are part of the regulatory documents, they must be maintained in close proximity to the area where the study drugs are stored or dispensed, available for inspection when the study is monitored or audited [216].

4.3.1.4. Patient Instructions

The investigator should explain the correct use and storage of IMP to each patient enrolled in the study at the start of the trial, and should also check, at appropriate intervals, that each patient is following the instructions properly. The patients should also be instructed to return all unused supplies, including empty containers at each visit. The supplies should be reconciled against the supplies dispensed to the patient and the supplies used by the patient, according to the sponsor requirements. The sponsor company should provide a drug-dispensing log and the investigator or pharmacist should completed it at each time that supplies are dispensed or returned.

Patients must comply with investigator's instructions by taking the recommended number of doses at the correct times, coming to the clinic as directed, being cooperative and honest with the staff who are conducting the study, and adhering to all other aspects of the study, such as, for example, avoiding the forbidden concomitant medicines and following the recommended diet [217-219]. Table 14 shows some examples of elements for patient instructions.

Especially if supplies are dispensed to subjects for self-administration (ambulatory), methods to ensure compliance (e.g., diary cards, instructions on labeling, supervised administration) and check compliance (e.g., tablet counts, plasma/urine assays, diary card review) must be in place [209, 220]. At each visit, the study subjects should be asked to return all unused supplies and empty containers to the investigator, which will be checked for compliance assessment. In the monitor's visit all relevant documents will be reviewed (e.g., case report forms (CRF) medication/device inventory, dispensing forms) to ensure that the data in the CRFs reflect the subject's compliance with the study [209].

Table 16. Examples of Elements for Patient Instructions

Patient Instructions	Name of medication
	Medication dosing and scheduling
	Any special storage instructions for the investigational drug
	List of medications that must not be taken concurrently
	List of any foods that must not be eaten
	Reminders to return all medication vials or blister packs, even if used or empty (for drug accountability and compliance assessment)
	Reminders not to share the medication with anyone else and to keep the medicine in a secure place.

4.3.1.5. Return and Destruction of IMP

The final step in the drug accountability process is the overall reconciliation of supplies and destruction. The reconciliation of initial inventory and the final returns must be undertaken, and any discrepancies, and reasons for any non-returns, must be explained and reported to the sponsor company [208, 221]. This process requires a lot of attention, as well as drug dispensing, since inadequate drug accountability belongs to the list of the most common findings observed during recent inspections [222]. All unused and returned medications or empty containers, must be stored securely at the study site until retrieved by the monitor [209]. In addition, the records should distinguish between unopened (not dispensed) and unused (by study subjects) supplies, identify broken or lost supplies, and show 95%-100% of supplies are accounted for [208, 216]. The study monitor will check the returned supplies and verify that they reconcile with the written specifications [209].
Final disposition and destruction must be carefully documented to allow assessment of possible detrimental environmental impact [209].

Destruction of returned IMP by the sponsor or CRO may not take place until the final report has been prepared. Currently, specialists advise that returned medication should be destroyed throughout the clinical study duration. The destruction process must be clearly documented and details of the method of destruction explained. However, in exceptional cases, unused study medications, such as cytotoxic and radio-labeled products, may be destroyed at the site, with appropriate documentation [209].

4.3.2. Computerized Systems in Clinical Trial Supply Chain: IVRS/IWRS

A large proportion of the cost of implementing a clinical trial is attributable to the production and management of IMP [223]. As a result, the management and distribution of IMP by computerized systems, in association with a telephone (IVRS) or web interface (IWRS), has becoming increasingly common [223]. For example, IVRS is a system that enables real-time monitoring of inventory in the clinical trial supply chain [183, 224, 225]. Such systems have been developed further into interactive web based systems (IWRS) utilizing the internet [226]. Both, IVRS and IWRS were developed to facilitate overall drug management and expanded to assist with dose titration, unblinding and expiry date update [226].

IVRS use the telephone as a means of inputting data, where pre-recorded prompts, that list the various options available or that request responses to particular questions, are played for the user. Data are entered and written to the underlying databases by using the telephone touch-tone keypad [225].

In an IVRS, medication packs are uniquely numbered not by subject number, but by medication pack number [225, 226]. Any pack can be assigned to any subject within the same treatment group. Rather than containing the entire medication supply for an individual subject within a pack, optimal application of this approach involves packing medication in sufficient packs to treat a subject for a complete dispensing interval. Because complete dispensing units may be allocated to other subjects in the same treatment group, using IVRS can reduce wastage of medication that would traditionally occur when a subject withdraws from the study [226].

Figure 13 illustrates how an IVRS is used to dispense medication packs to subjects, and how to maintain appropriate stock levels at site.

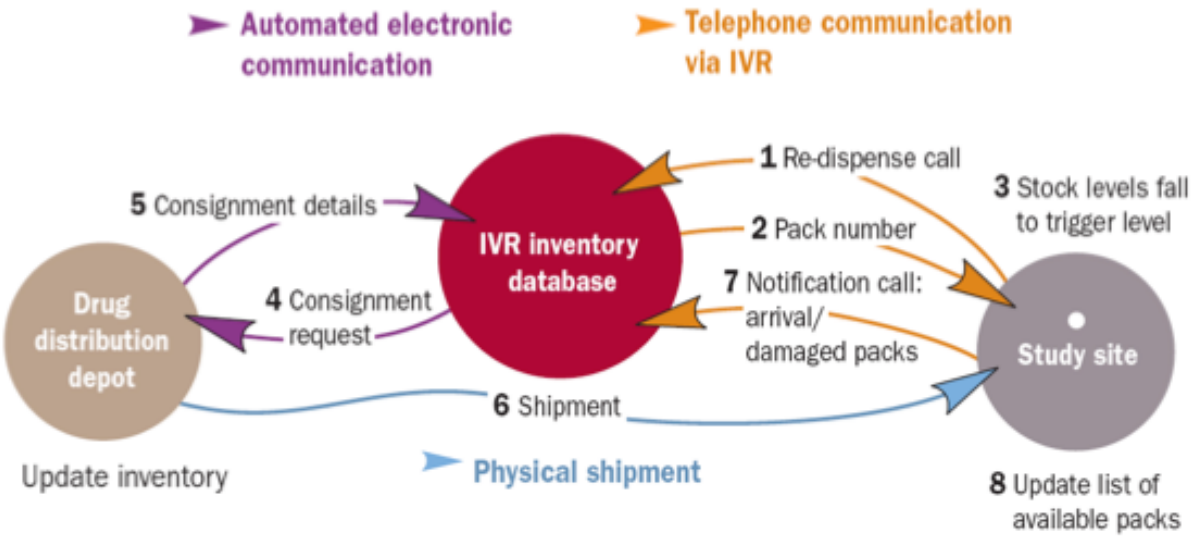

Figure 15. Medication Dispensing and Automated Site Inventory Control Using IVRS. Adapted from Byrom (2002) [225]

This figure shows the interaction of three main sites - the IVRS inventory database, the drug distribution depot (a pharmaceutical company or an external packing and distribution agency), and the study site. When an IMP arrives at a site, the site investigator or coordinator makes a call into the IVRS, indicating that want to dispense medication and identify the subject that requires medication. The system reports the medication pack number that should be dispensed to that subject, with reference to the known site inventory. An automated fax or e-mail confirms this information. One of the advantages of using a computerized system to control dispensing of IMPs is the ability to maximize the use of study supplies by allocating packs in expiry date order [224, 225].

For example, the IVRS dispenses the current medication pack, identifies that the stock at the site for that treatment has fallen to a predefined minimum level (trigger level), and sends an electronic request to the drug distribution depot for a consignment of additional supplies to be sent to the site. This request lists the number of packs of each treatment that should comprise the consignment. Sometimes the IVRS will dictate the pack numbers that

should be sent; other times the drug distribution depot will report back electronically which pack numbers were issued in the consignment. Then the medication is shipped to the site, where the CRC or pharmacist makes another call into the IVRS to register the consignment's arrival. Missing or damaged packs can be identified during this call, possibly triggering an additional consignment. Finally, the IVRS database updates the study site inventory to include the new packs that are then available for dispensing to future clinical trial subjects [225].

Interactive voice response systems represent a valuable tool to the clinical study manager, not only to perform randomization, emergency code-break, and medication supply management, but also to collect subject-recorded data, both during the study and in the study pre-qualification activities during the recruitment period [225, 227]. However, for studies using a simple design and where the availability or the cost of the drug is not an issue, then IVRS would not be appropriate [181, 226].

CHAPTER 5

Concluding Remarks

The preparation of an investigational medicinal product involves a multidisciplinary team and all efforts should be made to keep the same goal in mind. In fact, nowadays, developing drugs or devices for medical use is an expensive and lengthy business. It may cost several billion US dollars, and take more than 12 years to develop one single licensed drug, but efforts have been done to reduce development time, mainly through the contribution of high-throughput screening strategies, that improve the selection and optimization of molecules able to interact with high affinity and selectivity to the molecular target. Reduce the costs and time in the drug development is the most important challenge that pharmaceutical industry has to deal with.

During the development of IMP, special attention should be given to the solubility, as well as bioavailability assessment and bioequivalence studies, since they are the most challenging steps in the early phase of preparation of IMPs. Concerning the IMP production, the critical aspects, such as comparators, blinding and package, will determine the success of the entire clinical trial. Finally, when the IMP is fully prepared, it enters in the different clinical trials phases, with the aim of providing a range of information, such as efficacy and safety, which will determine its market introduction, bringing benefit to health. This whole process must meet a series of requirements previously established, and adequate trained staff, in order to minimize the costs associated with the development of the IMP, as well as accelerate its market entry.

The knowledge and training in the pharmaceutical medicine has been a significant contribution to the new era in drug development. Therefore, this work may help new students and new professionals, bringing some knowledge about the preparation and distribution of the investigational medicinal products.

However, it is relevant to point out certain limitations. This work addresses two major chapters: the preparation of the investigational medicinal products and their handling during the trial. Although there is a wide range of information on the preparation of

investigational medicinal products (pre-formulation, formulation, packaging, labeling, etc.), little information is available on logistics. Actually, there are several guidelines on the distribution and good clinical practices, but actual cases that describe the practice of procedures are hard to find in the available recourses. It was intended to summarize the process of preparation of medicinal products, addressing only the most relevant topics. However, in a future work it would be interesting to explore deeper the distribution of investigational medicinal products, addressing key findings of audits and find tools and procedures to avoid noncompliance.

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